A PRELIMINARY STUDY OF COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN-CALORIE MALNUTRITION

THESIS

DOCTOR OF MEDICINE

[PAEDIATRICS]
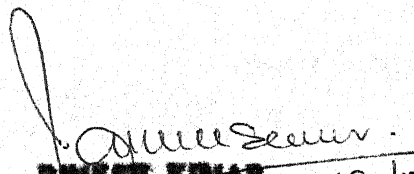
BUNDELKHAND UNIVERSITY
JHANSI, UTTAR PRADESH

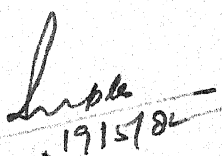1983   ANIL KUMAR

CERTIFICATE

This is to certify that the work entitled "A PRELIMINARY STUDY OF COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN - CALORIE MALNUTRITION" has been carried out by Anil Kumar, under my direct supervision and guidance in the Department of Paediatrics, M. L. B. Medical College, Jhansi.

RAMESH KUMAR
M.D.(Paed.),D.C.H., 19/5/82
Professor and Head,
Department of Paediatrics,
M.L.B. Medical College,
JHANSI(U.P.)

(CHIEF SUPERVISOR)

CERTIFICATE

Certified that the work conducted by Anil Kumar, entitled 'A PRELIMINARY STUDY OF COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN-CALORIE MALNUTRITION' was carried out under my supervision and guidance, by the candidate himself.

R.K. GUPTA
M.D.(Path.),
Reader,
Department of Pathology,
M.L.B. Medical College,
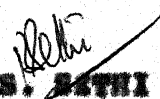JHANSI (U.P.)

(CO-SUPERVISOR)

CERTIFICATE

Certified that the work conducted

by Anil Kumar, entitled 'A PRELIMINARY STUDY OF

COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN-CALORIE

MALNUTRITION' was carried out under my supervision

and guidance, by the candidate himself.

R.S. RATHI
M.D. (Paed.),D.C.H.,
Lecturer,
Department of Paediatrics.,
M.L.B. Medical College ,
JHANSI (U.P.)

(CO-SUPERVISOR)

ACKNOWLEDGEMENT

ACKNOWLEDGEMENTS

' Nation marches on the tiny
feet of little individuals,
and hence no nation   can
afford to ignore its child-
ren'

                    - Jawaharlal Nehru

C O N T E N T S

ooo

INTRODUCTION

A cornerstone of the present century lies in the enormous advances made by umpteen workers in the field of immunology. Although the immunological profile of a host of Paediatric diseases has been studied so far, the immunologic study of 'Protein-Calorie Malnutrition' holds a special significance owing to its devastating effects on the growth and development of the child.

Nutrition is an essential part of the mosaic of factors that determines the natural history and biological gradient of the disease, especially in the developing world. In no other area this statement is more dramatically illustrated than in the interaction of malnutrition and immunity.

In India, children below 14 years of age constitute 42.8% while those below 5 years constitute 17% of the total population. Pre-school children not only form the bulk of child population but this period of childhood, especially the second year of life, is notoriously fraught with risk. The young child is "transitional" as regards diet, immunity to infections and psychological dependence. It is at this stage of rapid growth, exploration and interaction with the environment that a child is prone to encounter accidents, develop malnutrition and infections and suffer from behaviour problems.

1

Protein-calorie malnutrition (PCM) is the commonest child health and social problem affecting vast areas of the world. Obviously the condition is more prevalent and endemic in developing and under developed countries. PCM covers the whole range of mild to severe, classifiable and unclassifiable manifestations of malnutrition, including the two main clinical syndromes of kwashiorkor and nutritional marasmus. One important consequence of PCM is the retardation of child's growth and development. Mortality in children, especially among pre-school children, is closely related to nutritional status.

Nutrition, immunity and infection are known to be closely linked. Inadequate nutrition can alter the immunocompetence and thus increase susceptibility to infection. Infection, inturn, can adversely affect nutritional status.

Children with PCM are known to be unusually susceptible to infections, which are often more severe and slower to resolve, than is the case with those having normal nutrition. This has been attributed mainly to their altered immunocompetence. PCM causes depression of several host defense mechanisms including phagocytic and killing functions of leucocytes, cell mediated immune response, inflammatory response and antibody production.

The role of the complement in host defence mechanisms is well established. It is one of the principal mediators of the immune response and is capable of causing lysis of cells, bacteria and viruses. The complement system comprises a series of the proteins which require sequential activation for the biological action. Genetic deficiencies of the complement proteins are known to be associated with recurrent infections.

Recent reports have suggested that PCM adversely affects complement system, which may account in part for the increased susceptibility of malnourished children to infections.

It is in the light of these observations that the present venture though a humble one is directed at studying immunological profile of malnourished children. The study aims at the following :-

1-   To evaluate the complement system in pre-school children having protein-calorie malnutrition, so as to assess their immunological status in relation to the severity of malnutrition.

2-   To ascertain possible inter-relationship, if any, between the clinical progress of case and subsequent change in complement activity.

...

REVIEW OF LITERATURE

PROTEIN-CALORIE MALNUTRITION :

Historically, marasmus (Greek marasmos, wasting) was recognised for hundreds of years as being, with gastro-enteritis, a major contributor to high infant mortality. In the early part of this century reports from central Europe of so called 'Starch dystrophy' attracted little attention. The classic description by Williams (1933) of a disease attributable to protein deficiency, which she named 'Kwashiorkor' (taken from the 'Ga' language of Ghana), recognised that this was the disease, the first child got when the second was on the way. It was characterised by skin and hair changes, oedema, moonface, fatty liver, hypoalbuminaemia and psychomotor changes. Clinical descriptions of a disease, obviously similar, appeared from many other countries although in the West Indies for example, the dermatosis was uncommon, oedema prominent and the term 'Sugar baby' was subsequently used by Waterlow (1948) and Jelliffe et al (1954).

Jelliffe (1959) coined the term 'Protein-Calorie Malnutrition (PCM)' of early childhood to include the mild and moderate degrees and all the clinical types of the severe degree of malnutrition. Using a variety of biochemical tests McLaren et al (1967) were able to show that the severe degree of PCM in its various clinical forms of marasmus, marasmic-kwashiorkor and kwashiorkor formed a spectrum of both clinical signs and biochemical changes; both being most marked in full blown kwashiorkor and least evident in pure marasmus.

There was a short lived effort through World Health Organization (WHO) to introduce the term 'Protein-calorie deficiency diseases' but this was abandoned by the expert group meeting in 1970 in favour of PCM. Since then, the impact of proposals to replace the term calorie by Joule as a unit of energy measurement has led to a general use of the word 'Protein-Energy Malnutrition' rather than Protein-Calorie Malnutrition. At present there is an increasing recognition of the fact that the major problem all over the world is deficiency of food intake in general (and therefore of energy) rather than of protein in particular. Again, to emphasize that this is but part of the overall energy crisis of mankind the term energy-protein malnutrition or EPM has been used by workers to give the needed stress to energy deficit (McLaren, 1976).

Magnitude of the Problem :

PCM is one of the world's major public health problem especially in the underdeveloped and developing countries. Rao et al (1969), in a study of pre-school children of rural communities, found that the percentage prevalence of frank cases of kwashiorkor and marasmus were 0.6 and 1.0 respectively. In a survey of rural pre-school children Ghai et al (1970) reported that about 18 percent of all cases were undernourished. Out of these 1.7 percent had nutritional marasmus and 0.9 percent suffered from kwashiorkor.

Point prevalence figures have been collected
for a number of years by WHO. Bengoa (1974) reported
data from 77 nutrition surveys in 46 developing countries,
totalling near 2 lakhs children mostly under 5 years of
age and suggested that about 100 million children through-
out the world were suffering from moderate or severe PCM
at any one time. Bistrian et al (1974) have brought
attention to the large number of patients with secondary
malnutrition who were present in the wards of United
States hospitals.

Gopalan (1974) computed that nearly 65 percent
of toddlers in poor communities in India suffered from
moderate malnutrition and 18 percent from severe mal-
nutrition. Ghai (1975) analysed severe cases of PCM in
hospitals and reported 6.6 percent deaths in cases suffer-
ing from marasmus and 33.3 percent in kwashiorkor and
marasmic-kwashiorkor.

Ghai (1977) showed that malnutrition was a
major contributory cause of mortality in about 40 percent
of childhood deaths, even though it was often not listed
as a primary cause of death in most of studies. Rao (1975)
reported that marasmus and kwashiorkor, were seen only in
1-5 percent of the pre-school child population. As many as
60-70 percent of the children, on the other hand, suffered
from mild and moderate degree of PCM.

Recently Ghosh (1981) has shown that in India, there were about 100 million pre-school children out of which 3 to 4 million suffered from severe types of malnutrition, and probably 1 million of them died.

Classification (Grading) :

Grading of PCM is necessary for formulating therapy in individual patients and defining priorties for combating malnutrition. Three main direct methods of assessing PCM in the community have been used - clinical, anthropometry and biochemical.

Gomez et al (1955) is credited with the first ever classification of malnutrition, using the actual weight expressed as a percentage of standard weight (Boston 50th percentile) for age. The presence or absence of clinical characteristics such as oedema was not taken into account by the authors.

GOMEZ CLASSIFICATION

Grade of malnutrition	Weight
Normal	$> 90\%$ of expected weight for age.
Mild (1st degree)	89 - 75 %
Moderate (2nd degree)	74 - 60 %
Severe (3rd degree)	$< 60\%$

In a later modification, Jelliffe (1966) included all cases with nutritional oedema, irrespective of weight, in 3rd degree.

Although weighing scales may not always be available or maintained or used correctly and age is often not known accurately, yet this method is in common use. Its main drawbacks are, that it assumes all children of certain age to have the same weight, irrespective of their size as measured by height for example. It also includes such children who are underweight as a result of malnutrition in the past.

McLaren et al (1967) introduced a simple scoring system for classifying the severe forms only (satisfying Gomez criteria of weight $\angle$ 70%), based on all three methods of assessment viz. clinical, anthropometric and biochemical.

McLAREN CLASSIFICATION

Signs present			Points
Oedema	...	...	3
Dermatosis	...	...	2
Oedema plus dermatosis		...	6
Hair change	...	...	1
Hepatomegaly	...	...	1
* Serum albumin	(Total serum proteins)		
(g/100 ml)	(g/100 ml)		
$\angle$ 1.00	($\angle$ 3.25)	...	7
1.00 - 1.49	(3.25 - 3.99)		6
1.50 - 1.99	(4.00 - 4.74)		5
2.00 - 2.49	(4.75 - 5.49)		4
2.50 - 2.99	(5.50 - 6.24)		3
3.00 - 3.49	(6.25 - 6.99)		2
3.50 - 3.99	(7.00 - 7.74)		1
7/ 4.00	(7/7.75)		0

Score = Sum of points ; 0-3 = marasmus;
4 - 8 = marasmic-kwashiorkor ; 9 - 15 = kwashiorkor.
* Either serum albumin or total serum proteins were used for assessment.

This system has been used by a number of centres and is the only method available at present for a fairly precise and objective classification of the type of patients studied in hospitals. The problem of expressing chronicity and stage of disease however remains unsolved.

Some classifications have been designed to use measurements requiring only simple apparatus, avoiding the necessity for calculations and also the need to know the age of the child. These could be thus applicable under routine field conditions by unskilled personnel. Among these, Quaestick (Arnhold, 1969) method uses the height and mid-arm circumference. Based on this classification children were divided into two broad categories, 'malnourished' and 'normal'.

The ratio of mid-arm circumference/head circumference was shown to be independent of age at least from 3 to 48 months and was similar in either sex (Kanawati and McLaren, 1970). Based on this ratio, the following classification has been proposed to detect cases of malnutrition.

Ratio	Classification
$>$ 0.310	Nutritionally healthy.
0.310 - 0.280	Mild PCM
0.279 - 0.250	Moderate PCM
$<$ 0.250	Severe PCM

However, it needs to be emphasized that the method is rough, should not be used for individual children and is meant to screen large numbers.

The classification that appeared in the 8th report of FAO/WHO Expert Committee (1971) is one that was originally prepared by the Wellcome Trust and is sometimes referred to as the 'Wellcome' classification.

WELLCOME CLASSIFICATION

	Body weight as % of standard*	Oedema	Deficit in weight for height**
Underweight child	80-60	0	Minimal
Nutritional dwarfing	$\angle 60$	0	Minimal
Marasmus	$\angle 60$	0	++
Kwashiorkor	80-60	+	++
Marasmic-Kwashiorkor	$\angle 60$	+	++

* Standard taken as 50th percentile of the Harvard values.

** Weight for height = $\dfrac{\text{Weight of patient}}{\text{Weight of normal subject of same height}} \times 100$

'Wellcome' classification was probably the first in which an attempt was made to use weight/height as well as weight/age ratios and included a separate category of 'nutritional dwarfs'. However, it has some notable deficiencies. It confuses between the type and severity

of malnutrition. Diagnosis of marasmus, marasmic-kwashiorkor, and kwashiorkor refer to differences in the type of malnutrition and all are of similar severity. Thus in this system kwashiorkor appears to be less severe than the other two types as the body weight is 60-80 % of standard and not below 60%. Gradation of deficit in weight for height by such terms used as 'Minimal' and '++' can not be quantitated.

Nutrition Sub-Committee of the Indian Academy of Pediatrics (1972) classified PCM into 4 grades using 50th percentile of Harvard growth standard as a reference point.

CLASSIFICATION OF INDIAN ACADEMY OF PEDIATRICS

Grade of malnutrition	Weight expressed as percentage of reference standards.
I	71 - 80 %
II	61 - 70 %
III	51 - 60 %
IV	$\angle$ 50 %

Grade I and II are underweight and grade III and IV correspond to marasmus. When nutritional oedema is present, letter 'K' is suffixed to the grade denoting malnutrition, eg, 1K; 2K etc. 1 K and 2 K will mean kwashiorkor and grade 3 K and 4 K will correspond to marasmic-kwashiorkor.

Waterlow and Rutishauser (1974) published a
classification based on weight and height, thus taking
into account the effect of past as well as present
malnutrition. The 'present' malnutrition was called
'Wasting' as measured by loss of weight in relation to
height, and 'past' malnutrition called 'Stunting' was
seen as low height for age ratio.

WATERLOW AND RUTISHAUSER CLASSIFICATION

Grade	Stunting (height for age)	Wasting (Weight for height)
0	7 95%	7 90%
1	95-90%	90-80%
2	89-85%	80-70%
3	∠ 85 %	∠ 70 %

Waterlow maintained that weight/height was independent
of age, basing his argument on two sets of data which
were collected on children age between one and four
years.

MALNUTRITION, INFECTION AND IMMUNITY :

Scrimshaw et al (1968) reported that the
nutritional status was a critical determinant of
susceptibility to infection. He supported his clinical
impression by epidemiological data and experimental
studies in laboratory animals.

Philips et al (1968) also found that children with PCM were unusually susceptible to severe infections and took longer time to combat such infections.

Ramalingammani and Ramalingammani (1973) observed that malnutrition and infection, singly and in combination, contributed significantly to morbidity and mortality of infants and children in the developing countries.

In another comprehensive study, Reddy et al (1976) concluded that nutrition, immunity and infection were closely linked. They showed that inadequate nutrition could alter the immunocompetence, thus increasing the susceptibility to infection, and infection in turn, adversely affected nutritional status.

Defence Mechanisms in PCM :

In defence against bacteria, viruses and other pathogens, several facets of immunocompetence come into play. Phagocytic activity and bactericidal competence of leucocytes constitute the first order of defence. In addition, two other types of immune mechanism, which operate against infection, are the humoral and cell mediated immunity. Also, there are other nonspecific defence factors such as lysozyme, complement and opsonins which play an important role in determining resistance to infection. Alterations in one or more of these mechanisms may be expected to increase susceptibility to infections.

PCM causes depression of several defence mechanisms.

Smythe et al (1971) demonstrated profound depletion of the thymolymphatic system and severe depression of cell mediated immunity in malnutrition.

Chandra (1972) noticed that antibody response to tetanus toxoid was adequate, but response to S. typhi vaccine was significantly reduced in malnourished children. He also reported depressed cell mediated immune response in PCM.

Selvaraj and Bhat (1972) and Seth et al (1972) showed that phagocytic and killing functions of leucocytes were decreased in children with PCM.

Edelman et al (1973) observed depressed inflammatory response and cell mediated immune response in PCM.

Reddy et al (1977) showed that both the cell mediated immune response and antibody response to bacterial antigens were impaired in children with severe PCM. However, the immunological responses were not altered in those with mild to moderate PCM as observed by authors.

Kumar et al (1978) observed depressed cell mediated immunity in children with PCM.

Puri et al (1980) reported that various parameters of cellular immune response were significantly depressed in severe PCM. However, the authors also

observed that humoral immunity was not altered in PCM except in the presence of infection, when there was some increase in IgG levels.

COMPLEMENT SYSTEM :

Complement is a system of factors occuring in normal serum which are activated characteristically by antigen-antibody reaction and subsequently mediate a number of biologically significant consequences. It is now apparent that complement acts as the principal mediator of the inflammatory response and plays an essential role in host defences against infection.

According to McConnell and Lachmann (1976) and Fepra (1976), role of complement has advanced in recent years from being a collection of abstruse biochemical phenomenon to a system which has fundamental importance in immunogenetics and immunopathology.

History :

Pfeiffer (1894) demonstrated that the immune system of guinea pigs acquired the capacity to dissolve cholera bacteria (Pfeiffer's phenomenon).

Bordet (1896) repeated the experiment and found that Pfeiffer's phenomenon required two components of serum : a heat stable component (stable at 56°C for 30 minutes) that was present only in immune serum and a heat labile component present in immune as well as nonimmune sera. Bordet described the same phenomenon

in the serum of animals immunised with red blood cells of different species and called heat labile factor 'Alexin'. The term alexin was later replaced by the new term 'Complement' proposed by Ehrlich and Morgenroth (1899). These authors concluded that serum contained two substances : the interbody having two haptophore groups (analogous to immune body) and an addiment, which they named complement because it completed the antibody's immune response after it reacted with antigen.

By the 1920s there were 4, by the 1960s, 9 components were known (one of which had 3 subcomponents). Austen et al (1968) labelled the original system of 11 interdependent factors as the classical pathway of complement.

Gotze et al (1971) and Gewurz (1972) described a second major pathway of activation of complement, the alternative or properdin pathway. Authors also reported that this system consisted of at least 4 factors.

Basic Precepts :

Johnston and Stroud (1977) described the basic precepts of complement system :

1. Complement is a system of interacting proteins. The biologic functions of the system depend upon the interaction of individual components.

2. The components interact in an orderly, sequential fashion. This has been referred to as 'Cascade', in that activation of each component (except the first) depends upon activation of the prior component or components in the sequence.

3. Interaction occurs along two pathways :

The Classical Pathway - in which the components interact in the following order : Antigen-antibody C142356789, and the more recently discovered alternative or Properdin Pathway. In this alternative pathway the chain of reaction is : Activator (antibody) -- Properdin system -- C356789. Whether an antibody is required and what is the exact sequence of interaction of components in the alternative pathway is still not clearly understood.

4. The interaction of early acting components (C14235) is enzymatic in nature, so that "activation" refers to transformation of the components into an active enzyme. In contrast, the interaction between C5b, C6, C7, C8 and C9 is non-enzymatic through non-covalent, probably hydrophobic, bonds. In the case of C1, activation is a result of its interaction with antibody. Activation of C4, C2, $C3$, C5, as well as of factor B of the alternative pathway, is secondary to cleavage by a preceding component or components. This activation of early components generates an enzyme which fixes to the antigen-antibody complement complex and catalyses a reaction on the next component, whereas later acting components (C6 to C9) adsorb on to the complex or the underlying cell by an interaction which depends on a change in their configuration.

<u>Sequence of Activation</u> :

Johnston and Stroud (1977) summarised the sequence in which the components of the classical pathway and alternative pathway interacted. The interdigitation between classical and alternative pathways and the classical and functional by products of these reactions were also described (Fig.: 1, 2).

<u>THE BIOLOGICAL ROLE OF THE COMPLEMENT SYSTEM</u> :

Fust (1978/1979) inferred that the complement system played an essential role in a number of physiological processes participating in the defence mechanism of the organism and were mostly favourable. However, he emphasized that like other plasma enzyme systems, complement played a dual role. He expressed the opinion that events occuring during complement activation and the substances liberated during such activation could induce pathological processes. To substantiate his opinion, author quoted the example of complement playing an essential defensive role in the elimination of immune complexes (ICs) while it also caused tissue destruction.

<u>Participation in Host Defence</u> :

Dias da Silva et al (1967) and Shin et al (1968) expressed that complement was a dominant force in mediating inflammation, phagocytosis and cytolysis. They showed that when the functional unit was activated, both C3a and C5a allowed histamine release from mast cells

The Complement System

Classical pathway

(C~CRP)

$\overline{C1}$ ... $\overline{C1}$ ... C4 → $\overline{SAC1b}$ ... C2 → $\overline{SAC1b2}$ → $\overline{SAC1b}$

Adherence

'C-kinin'

C4a

(C1 INH)

requires Ca^{++} & Mg^{++}

Alternative pathway

Polysaccharides
LPS
IgA
CoVF

Properdin System
(P)
C3bBb
Properdin
Factor D
Factor B

C3b	INA
	βIH

C3b amplification
C3a anaphylatoxis
Enhanced Phagocytosis
Immune adherence
Binding to B cells (C3b, C3d)

C3a anaphylatoxin
chemotaxis

C3

C5

C5a $\overline{SAC1b}$

C5b	INA
	βIH

C5a chemotaxis

$\overline{SAC1b}$ C5

C6
C7 → C 567

Reaction with Bystander cells
Chemotaxis

$\overline{SC567}$

C8
C9 → $\overline{C5b9}$

Cytolysis

Fig. 1 : Scheme of action of the complement components of the classical pathway and interaction with the properdin system.

E = Erythrocyte (could stand for any antigen 'Ag' eg. bacterium, virus, tumor cell); A = Antibody (Ab) (of IgG or IgM classes only);
C-CRP = C – Carbohydrate – C-reactive protein; C1 INH = C1 inhibitor;
C3b INA = C3b inactivator; Ig = Immunoglobulin; LPS = Lipopoly-
saccharides.

Fig. 3 : Interaction of components of the
alternative (properdin) complement
pathway; Ab = Antibody; Ag = Antigen.

and basophils. Besides, there were muscle contractions,
an increase in capillary permeability and leucotaxis of
neutrophils, eosinophils and mononuclear cells.

Rother (1972) reported a small molecular weight
cleavage fragment (C3a?) from C3 which was responsible
for release of neutrophils from bone marrow.

Complement forms a vital link in host resis-
tance to infection. During infection with bacteria,
parasites and yeasts, the processes of cytolysis and
phagocytosis occur. Allison (1974) observed that with
viral infections, cytolysis and phagocytosis did occur
but in addition, complement also participated in the
process of neutralization. Author described that this
process either prevented the virion from entering into
the target cell or that it interfered with the replication
of virion inside the cell.

Strauss et al (1975) stressed that C3 activation
might be important for initiating oxidative metabolism of
the polymorphonuclear leucocytes and the release of
lysosomal enzymes.

Koopman et al (1976) reported that by interaction
with B type lymphocytes, C3b mediated the release of
chemotactic factors from macrophages, B cell proliferation
and formation of antibody.

Miller et al (1976) observed that neutralization
of virus required either the deposition of C1, C4 and C2
or the fixation of C3 on to the virus. Depending on the

type of virus, activation and deposition of C3
could occur by the classical or alternative pathway.
Antibody was not necessarily required in complement
activation since some viruses could directly activate
C1 by contact, as observed by the authors.

Spitzer (1977a) reported that besides
providing for various elements of inflammation,
complement was also involved in a direct attack on
pathogenic agents by cytolysis, the entire functional
unit being necessary for this purpose whether through
classical or alternative pathway. Author also showed
that by the deposition of C3b on the surface of the
offending organism, complement system promoted
phagocytosis by providing a contact point between
the organism and phagocyte thus allowing internali-
zation. This fact was further substantiated by
Johnston and Stroud (1977). Johnston and Stroud
(1977) summarized specific activities of the
complement system in host defense against infection
as follows :

ACTIVITIES OF COMPLEMENT IN HOST DEFENCE AGAINST INFECTION

Components or Fragments	Functional activity
C14, C1423	Virus neutralization.
C3a, C5a	"Anaphylatoxin" (Capillary dilatation)
C3 & C5 fragments, C567	Chemotaxis of PMNs, Monocytes, eosinophils
C3b	Opsonisation
C3b, C3d	Enhanced induction of antibody formation.
C3b	Stimulation of B-cell lymphokine production.
C3 Cleavage product	Induction of granulocytosis
C5	Opsonisation of fungi
C1-6 (?additional components).	Endotoxin inactivation
C1-9	Lysis of viruses, virus infected cells, tumour cells, mycoplasma, protozoa, spirochetes and bacteria.

Fust (1978/1979) suggested that alternative pathway represented the first defence line against bacterial infections; it was capable of reacting with bacteria, opsonising them and supporting their elimination before the specific antibody response would start.

The Role of the Complement System in the Elimination of
Immune Complexes (ICs) :

There are different complement mediated
processes which cooperate in IC elimination.

According to Gigli et al (1968) and Ruddy et al
(1972), the ICs, bearing C3b on surface, were capable of
binding to the C3b receptors of polymorphonuclear leuco-
cytes and also to the cells of the mononuclear phagocyte
system. Thus ICs were finally phagocytosed.

Miller et al (1975) reported that C3b in the
immune complex could change the conformation of the
complex itself. As a result, large complexes were split
into the smaller ones. These smaller complexes were
unable to deposit in the tissues and got ultimately
detoxified.

METHODS OF EVALUATION OF COMPLEMENT SYSTEM :

1. Functional Assessment :

Functional assessment of the activity of the
complement system is made by measuring the lysis of
antibody coated sheep erythrocytes (for total haemolytic
complement) or unsensitized rabbit erythrocytes (for
alternative pathway activity) by normal human serum.

1) Total Haemolytic Complement (CH$_{50}$):
The technique of determination of total haemolytic
complement (CH$_{50}$) was originated by Mayer (1961). He
observed that testing for total haemolytic complement
served as a useful screening procedure for most of the

diseases of the complement system. This assay depended
upon the ability of all 9 classical pathway components
to interact and lyse antibody coated erythrocytes.
Author concluded that the dilution of serum which lysed
50 percent of the cells, determined the end point and the
reciprocal of that dilution was the CH_{50} or "complement
haemolysis of 50% of cells."

Spitzer (1977b) reported that some of the
components might be reduced significantly in amount
without causing a noticeable deviation in the CH_{50}.
Thus with C3, for example, it took nearly a 50% reduction
to decrease the CH_{50} since C3 was normally present in
large quantities in serum. In view of this major dis-
advantage of this screening test, author inferred that
one could not place too much emphasis on this single
assay.

Interestingly, Johnston (1979) drew attention
and reported that in the congenital deficiencies of one
or more classical pathway components, the CH_{50} value
would be zero or almost so; values in acquired deficien-
cies would vary with the severity of the underlying
disorder. Author also strongly emphasized that this
procedure should be available as a screening test to
every physician.

2) Alternative Pathway Activity :

Platts-Mills and Ishizaka (1974) observed that
unsensitised rabbit erythrocyte (RRBC) activated the

alternative pathway of complement in normal human
serum. Hence authors used the lysis of RRBC to assess
the functional activity of the alternative pathway compo-
nents including (C3-C9) and it was expressed as AP_{50}.

3) Functional or Immunohaemolytic Assay for the
   Different Components of Classical and Alternative
   Pathway :

      Fust (1978/1979) described the use of
functional (immunohaemolytic) assay for the assessment
of different components of classical and alternative
pathway. In the procedure, great excesses of the
preceding components were added to sensitised sheep
erythrocytes. Then serial dilutions of the serum
(under test) were added to the system to serve as the
only source of complement component to be tested.
Finally the latter components were added. Author
showed that the concentration of tested component
could be calculated from the percentage haemolysis of
RBCs and the corresponding serum dilution. Titre of
the component was expressed in CH_{50}/ml units (CH_{50}
being the complement quantity causing lysis of 50% of
the erythrocytes).

II- Immunochemical Assessment :

      The immunochemical technique is an important
measure of quantitative assessment of various individual
complement components in the serum.

The double diffusion technique as derived
by Ouchterlony (1948) is a qualitative technique, used
to detect the presence of antigen or antibody in a
test solution and to show antigenic cross reactivity.
The author described that when antigen and antibody
were placed in wells cut in a gel and allowed to diffuse,
visible precipitin lines were formed at the zone of
equivalence. By a system of serial dilution of test
samples, an approximate concentration or titre could
also be derived, as reported by the author.

Grabar and Williams (1953) described the
technique of immunoelectrophoresis. Immunoelectro-
phoresis combined the advantage of zonal electrophoretic
separation of proteins and the immunological discrimina-
tion of double diffusion.

Mancini et al (1965) described Single Radial
Immunodiffusion, as a technique for quantitative esti-
mation of proteins (antigens). Authors showed that the
antigen diffused radially from the point of application
into an antibody containing gel and a circular
precipitate (ring) was formed at the zone of equivalence.
Keeping antibody concentration and gel thickness
constant; the area covered by precipitin ring was
proportional to the concentration of antigen. In the
original method, authors allowed the antigens to
diffuse at room temperature until the precipitin rings
stopped growing in size.

Fahey and McKelvey (1965) modified Mancini's technique of Single Radial Immunodiffusion. They reported that the readings could be taken after a fixed time viz. 18-80 hours; giving rise to only minute differences in the results.

Laurell (1966) described the technique Rocket Immunoelectrophoresis, a simple, quick and reproducible method for determination of a single protein in a protein mixture using number of samples simultaneously. Author applied diluted samples in wells side by side in a layer of agarose gel containing a nonspecific antiserum. The identification of the protein was given by the rocket-shaped precipitate formed and quantitation was done by measuring the height of the precipitate rocket or the area under it.

III- Demonstration of Complement Activation Products:

During last few years, direct methods for the demonstration of complement activation or breakdown products have become popular.

Lachmann and Coombs (1965) found that the titre of immunoconglutinin in serum, an antibody to reacted C3 and C4, was a measure of the extent of, in vivo, complement activation.

Thompson (1977) described a simple and reliable technique, known as 'Two Dimensional or Crossed Immunoelectrophoresis'. Author based this

tent on the principle that both C3 and its activated
fragment shared the same antigenic determinant but had
different electrophoretic mobilities.

IV- Miscellaneous Method :

Fust (1978/1979) observed that deposition of
complement components in various parts of renal
glomeruli was of special importance in the diagnostics
of certain renal diseases. Author also noticed that
these deposits were made visible by immunofluorescence.

SIMPLIFIED SCHEME FOR EVALUATION OF COMPLEMENT :

Spitzer (1977b) gave a simplified scheme for
evaluation of complement associated disorders by three
screening tests including CH_{50}, C3 and C4 assays (Fig. 3).

PCM AND COMPLEMENT :

Smythe et al (1971) were the first to call
attention to altered total haemolytic complement (CH_{50})
activity in infants with PCM. Estimation of haemolytic
complement by these authors showed that 61% of infants
with PCM had values well below 1/64 and 39% had values
within normal limits, whereas the controls were consis-
tently within a range of 1/128 to 1/512. These
differences were found to be statistically significant.

Chandra (1975) first estimated the serum
levels of complement component C3 in malnourished
children by Mancini's single radial immuno-diffusion

$CH_{50}, C4, C3$

| | CH_{50} ↑ NI C4 ↑ C3 | NI CH_{50} NI C4 C3 ↑ | | CH_{50} ↑ NI C4 NI C3 | | NI CH_{50} NI C4 NI C3 |

Classical Activation Unit and Functional Unit: CH_{50} ↑, C4 ↑ NI, C3 ↑

Alternative: Properdin activator or C1 by pass — Activator unit and Functional Unit or C3 deficiency (CH_{50}) ↑

Congenital deficiency of C4 or Malaria

Isolated complement deficiency

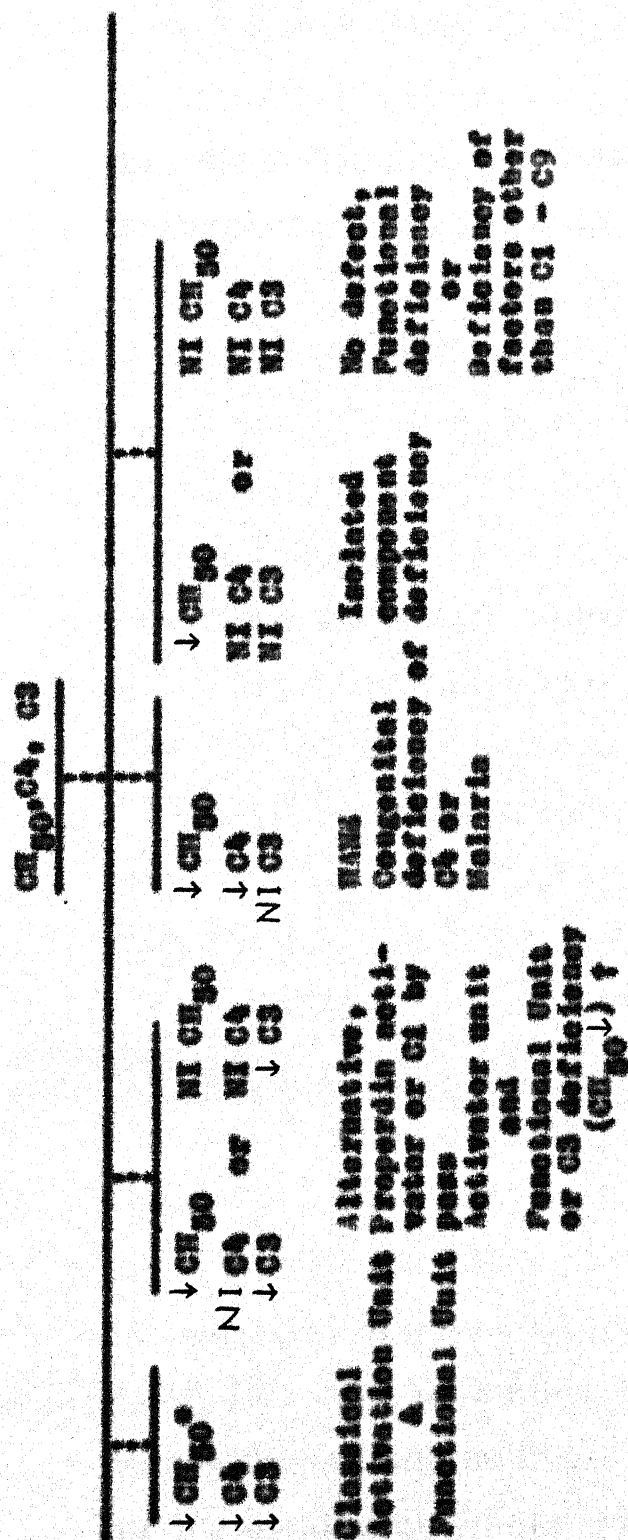No defect, Functional deficiency or Deficiency of factors other than C1 - C9

Fig. 3 : Simplified scheme for evaluation of complement-associated disorders; NI, Normal; *, Can be normal; ↑, differentiated by assays for C1, factor B, and properdin or properdin converten.

technique. Serum levels of C3 were significantly lower in the malnourished children (95 mg% ± 32 S.D.) than in the control subjects (136 mg% ± 31 S.D.). He also suggested that this could well be the result of reduced synthesis by the liver cells.

Sirisinha et al (1973) studied the serum levels of complement proteins C1q, C1s, C3, C4, C5, C6, C8, C9 and C3 proactivator (C3 P.A.) in twenty children with PCM on admission and at intervals thereafter during different dietary treatments and compared the results with those in sixteen normal children of the same age in the same geographical area. A majority of patients were judged to be infected on admission and were placed on antibiotic therapy. Authors also found that the serum levels of all the complement proteins except C4 were markedly lower in malnourished children than in the normal children, and children with kwashiorkor had lower complement levels than those children suffering from marasmus. Admission levels of 8 of the 9 components (except C4) were slightly lower in the severely infected compared with the mildly infected patients as observed by the authors. Further the difference between the two groups was less pronounced on day 8, when infection seemed to be under control. However, the differences in the complement levels between severely infected and mildly infected patients were smaller than those between the malnourished and the normal children. These results

suggested that the poor diet associated with impaired synthesis of complement components and to a lesser extent, infections led to reduced serum complement levels in untreated PCM children. During follow up the quality of dietary protein and the calorie intake had a pronounced influence on the repair of the complement system, the best response was obtained by high calorie (175 C/Kg/day) and high protein diet (4 g/kg/day). The levels of most complement components during treatment rose to above normal values. Mechanism of this 'overshoot' or 'rebound' should be due to accelerated synthesis after increased complement consumption in vivo as suggested by authors. They also suggested that, in addition to complement consumption, correction of impaired synthesis could result in complement 'rebound'.

In view of above observations, Chandra (1975) again subjected 25 children, aged 6 months to 4 years, having PCM to complement study and matched with 20 healthy controls. He observed that in 18 children there was clinical and microbiological or radiological evidence of systemic infection. These were treated with appropriate antibiotics. Three to eight weeks later a second sample of blood was drawn from 10 children, available for re-examination and no longer undernourished. He noticed that total haemolytic complement and C3 concentration were significantly decreased in

malnourished children than that of healthy controls.
There was also a significant positive correlation
between C3 concentration and CH_{50} activity ($r = 0.7131$).
Author observed a greater reduction in complement levels
in malnourished children with infection compared with
noninfected ones. However, in nutritionally normal
subjects, infection was associated with high C3 levels.
Author expressed the opinion that reversible but pro-
found disturbance of complement even in infected under-
nourished patients could be the result of at least two
factors. One, antibody synthesis and cell division
might get priority over complement synthesis in the
face of limited nutrient resources of the host.
Secondly, infection might be associated with complement
consumption. Presence of second phenomenon, operating
in these patients was supported by the fact that
electrophoretically altered forms of C3 in 14 cases
and raised levels of immunoconglutinin were detected
in most of the cases. Finally the author suggested
that reduced complement function in malnutrition was
the combined result of impaired synthesis, complement
activation in vivo, change in plasma volume, protein
losing gastroenteropathy, and that it might contribute
to an increased susceptibility to infection in under-
nourished individuals.

Neuman et al (1975) studied 76 malnourished
Ghanain children, aged 6 months to 6 years, and 41

age matched controls. Cases were divided into three
groups :-   Group I - Severely malnourished (34)
children whose weights were 51-60% of 50th percentile
of Harvard standard and /or serum albumin levels below
2.5 gm%. These children formed two subgroups -
Kwashiorkor (23) and marasmus (11). Group II - Moderately
malnourished, included 42 children whose weights were
61-80% of standard and serum albumin levels greater than
2.5 gm% and had minor skin and hair abnormalities. Lastly
Group III - Control group consisted of children whose
weights were 78% of standard, had normal serum albumin
levels and were free of chemical signs of malnutrition
or obvious infection except for pyoderma in a few cases.
However, intestinal parasites were found in most of these
cases. Authors noticed that levels of complement C3 were
significantly reduced in the severely malnourished group
as compared to the other two groups. Mean C3 levels in
group II and III were slightly reduced but in a low
normal range when compared to normal American children.
In group I, children with kwashiorkor had lower C3 levels
(mean $86 \pm^{5}$ mg/100 ml) as compared to children suffering
from marasmus (mean 71 ± 7 mg/100 ml). After 2 weeks of
nutritional therapy mean C3 level in group I children
with kwashiorkor increased to 78 ± 3 mg/100 ml. C4
levels were found to be normal in all 3 groups. Author
explained that decreased C3 levels could be due to
diminished protein synthesis by the liver as suggested

by a good correlation between the degree of C3 depletion
and severity of depletion of other proteins. They also
did not rule out the possibility of accelerated consump-
tion as a result of infection occuring in these cases.
Normal C4 level suggested that the alternative pathway of
complement was activated in malnutrition probably by
bacteria and their endotoxins which led to breakdown of
C3 and later components without affecting C1, C2 and C4.

Using the haemolytic complement (CH_{50}) assay,
Sankimd et al (1976) evaluated the complement system of
28 children with severe PCM during their hospital admi-
ssion and recovery. Children were classified clinically
as having marasmus (M), marasmus-kwashiorkor (MK), and
Kwashiorkor (K) based on the scoring system of McLaren
et al (1967). The mean CH_{50} activity in children with
kwashiorkor was significantly less on hospital days 1 and
4 than in 17 healthy control subjects. On day 8 it rose
to normal, and by day 50, it was significantly higher
than controls. The mean CH_{50} titre of 15 well nourished
febrile children was, in contrast to that of untreated
PCM, significantly greater than in the healthy controls.
Therefore it was unlikely that fever present in many
PCM children, lowered their CH_{50} activity. Among child-
ren with PCM, 11 (40%) had detectable serum anticomple-
mentary (AC) activity in their serum on either day 1 or 4.
Significantly, the CH_{50} titre in a PCM serum correlated
inversely with the amount of AC activity in the serum.

These results indicated that, in children with PCM, complement system was compromised functionally, and that its repair coincided with the intake of adequate diet. Further, presence of AC activity provided a possible explanation for depressed complement activity in some untreated PCM children.

Complement components C1-C9 were also estimated in children with protein-calorie malnutrition by Olusi et al (1976). Concentrations of C1q, C1s, C3, C6 and C9 were significantly lower in children with PCM, than in age and sex matched control children as observed by the authors. Children with marasmus tended to have higher values of these complement components than children with kwashiorkor. Complement C3 and C9 were the most severely affected by malnutrition and it would appear from the study, that more severe the degree of malnutrition, as judged by clinical examination and serum transferrin concentration, greater was the reduction in the serum concentration of C3 and C9. It was observed that the serum concentrations of C3 and C9 were lower in kwashiorkor and marasmic children with infections than in children without infections. There was no correlation between C3 and IgG concentrations as reported by the authors. It was suggested that probable responsible factors for reduced complement activity in malnutrition were reduced protein synthesis and increased utilization due to concomittant infections.

It was significant to observe in this series that there was no change in C4 concentration in children with malnutrition. It would appear that C4 was synthesised by the same cells responsible for the production of IgG and hence that there was a preferential synthesis of C4 and IgG in children with PCM. C5 was the only complement component which was significantly higher in malnourished children than in normal children, thus suggesting that this complement component was an acute phase protein. During refeeding, C3 was the first complement component to show a significant rise in concentration; this was followed by C9 and then C6. There was no change in C4 concentration while the levels of C5 fell. A conclusion drawn from these observations was that, of all of the complement components, C3 was the most sensitive index of nutritional status.

Kielmann et al (1976) carried out first ever study in nonhospitalised pre-school children in nine villages of the former Narangwal Rural Health Research Centre in Ludhiana District, Punjab. In these villages, all children upto 3 years of age routinely received curative and preventive medical facilities besides food supplement. Authors divided the children into 3 groups based on weight for age. These groups corresponded approximately to 80% or higher, 65% to 79% and less than 65% of the Harvard median, respectively. The children had significantly lower complement levels

(for all the three groups) as compared to those of
reference population of identical age distribution.
Children in the lowest weight for age group had less than
50% and those in the two higher nutritional groups had
between 60% and 70% of the complement levels as compared
to the reference population. Complement C3 levels were
also positively correlated with several other anthropo-
metric indices (weight-chest circumference and arm
circumference for age) as observed by the authors.

Spitzer (1977a) mentioned that in patients
with malnutrition, a consideration of failure of
synthesis of C3 might be entertained. Decreased C3
levels could be used for diagnosis and also for
monitoring during follow up.

Haller et al (1978) measured the plasma levels
of complement haemolytic activity (CH_{50}), of some comple-
ment components and of C3d, a C3 break-down product, in
59 African children with various types of PCM including
kwashiorkor, before and during recovery and compared them
with two control groups, each consisting of ten age
matched children and having a weight-age ratio of 7 90%
of the Harvard standard. One of the control group was
suffering from infection at the time of admission and
the other had none. A significant decrease of CH_{50},
C3, C9 and factor B was observed in PCM. The decrease
of CH_{50}, C3 and C9 appeared to be correlated with the
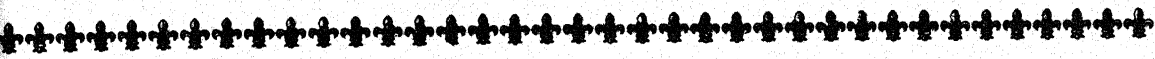severity of PCM, which was not the case for factor B.

On the other hand, levels of C4, C5 and C1 - Inactivator
fell within normal range. Increased plasma levels of
C3d with higher C3d/C3 ratio were also found in PEM
patients as compared to non-malnourished infected
patients and to normal non-infected children. Serial
measurements done during the recovery of PEM indicated a
progressive normalization of all complement values, as
well as a decrease of C3d/C3 ratio. Presenting their
conclusions authors thought that two mechanisms could
possibly be involved in the impairment of complement
system in PEM : (1) a decreased synthesis of at least
C3 and C9, as suggested by a significant correlation of
C3 and C9 levels with those of serum albumin and
cholinesterase; (2) an increased catabolism of C3,
possibly due to an activation of the alternative comple-
ment pathway, as suggested by the increased levels of
C3d and decreased level of factor B both of which were
significantly correlated with C3 levels but not with
albumin levels. Again C4 levels were normal as
observed by authors.

Keilmann and Curole (1979) observed C3 comple-
ment levels in 83 rural pre-school children of North
India. They related C3 complement levels to various
parameters of nutritional status and past episodes of
infections. All children were normally active and free
from intercurrent infections. Mean complement levels
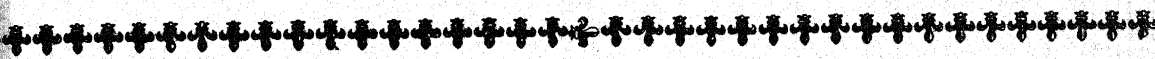were 20% lower than those found in an age-matched European

reference population. Low complement (C3) levels were associated mainly with children who were both stunted and wasted, as well as with those who had experienced frequent purulent skin infections in the past.

According to Johnston (1979) patients with malnutrition could have significant depletion of complement components and functional activity of complement. Although synthesis of components was depressed in malnutrition, serum from some patients also appeared to contain immune complexes which could accelerate depletion.

In a recent study Jagadeesan and Reddy (1979) reported that total haemolytic complement (CH_{50}) as well as C3 levels were significantly decreased in children with kwashiorkor (some of them had associated infections) and returned to normal after 2-4 weeks of treatment with protein and calories. In marasmic children, though the total complement activity was not significantly altered, C3 levels were reduced. However, neither CH_{50} nor C3 levels were found to be altered in mild to moderate protein-energy malnutrition (weight between 60-80% of standard). Reduction in serum complement activity could be one of the factors responsible for the frequent occurrence of infections in children with severe PEM as suggested by authors. Their study also indicated that immune status was not affected by milder degrees of PEM.

***

MATERIAL & METHODS

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College, Jhansi, in collaboration with the Department of Pathology, M.L.B. Medical College, Jhansi, over a period of 11 months from May 1981 to March 1982. Pre-school children (1-5 years age), attending the Well Baby Clinic and those admitted in the Paediatric ward, were selected for this study. Cases were grouped as :

A   Healthy normal controls.

B   Children suffering from protein-calorie malnutrition (PCM).

SELECTION OF CONTROLS :

Twelve normal healthy pre-school children were taken as control. Criteria for selection of control cases were :

1-   Weight more than 80% of the 50th percentile of Harvard standard for age.

2-   Exclusion of all possible factors known to affect the complement status viz. infections, liver and renal disorders, immunodeficiency diseases and corticosteroids.

SELECTION OF CHILDREN SUFFERING FROM PCM :

Thirty two children having PCM were taken for the present study. Criteria for selection of PCM cases were :

1.   Weight less than 80% of the 50th percentile of Harvard standard for age.

2.   Children having primary liver disorders,
      renal disorders and immunodeficiency diseases
      were excluded from the study.

3.   No case was receiving corticosteroids.

      Children suffering from PCM were treated with
broad spectrum antibiotics, intravenous fluids, supple-
mental vitamins and minerals as per the requirements.
All patients were put on nutritional rehabilitation
schedule to raise the daily intake of food to more than
100 calories/kg along with 3-4 gm proteins/kg of the
expected body weight.

      An attempt was made to follow the cases at 2
weeks, 4-7 weeks and 10-12 weeks interval.

      Besides name, age, sex, address and socio-
economic status following facts were recorded in each
case :

DIETARY HISTORY :

      Dietary history was recorded with special
emphasis on the following points :

a)   The age upto which breast milk was given.

b)   Age at which artificial milk was started.

      Type of artificial milk and the quantum of
dilution were also recorded.

e)   Age at which semisolids and solids were started,
      was also noted.

d) Present diet in terms of quantity and quality of food material used in feeding the child was recorded. Total calorie and protein intake per day were thus recorded in every case to ascertain the cause of PCM.

IMMUNIZATION STATUS :

History of immunisation was taken from the parents or family members. For small pox and BCG vaccination confirmation was made by careful inspection of scar marks. For polio and DPT vaccination, however, verbal statements were relied upon, confirmation was done by records, if available.

ANTENATAL, NATAL AND POSTNATAL HISTORY :

To rule out any secondary factor which could give rise to malnutrition, relevant antenatal, natal and post natal history was recorded. Special emphasis was also given to birth weight and gestational age of the child.

MILE STONES :

Mile stones were recorded under 4 headings : motor, manipulative, social and speech. The age, at which the child attained them was ascertained, by objective and subjective assessment.

PRESENT, PAST AND FAMILY ILLNESSES :

Present ailment relating to various systems were recorded.

Efforts were made to find out the occurrence of any acute or chronic illness in the past, that might have affected the nutritional status of the child. Past illness was mainly recorded in two categories : Category i - History of acute illness viz., fever, vomiting, diarrhoea and convulsion, lasting more than 4 days during the previous two weeks; category ii - History of cough, fever, vomiting, convulsions and diarrhoea, lasting more than 2 weeks any time during the previous 6 months. Besides these, definite history of primary complex, pertussis, measles or worm infestation was also recorded.

An enquiry was made about the history of any familial illness such as tuberculosis and diabetes.

PHYSICAL EXAMINATION :

A thorough clinical examination was made including those related to psychomotor changes, pallor, oedema, skin changes, hair changes, amount of subcutaneous tissue and muscle mass. Eyes were examined for the presence of xerosis and Bitot's spots. Skin was examined for any evidence of xerosis, hypopigmentation, hyper- keratosis and any dermatosis. Lips, gums and tongue were examined for the presence of angular lesions, cheilosis, gum swelling and glossitis.

Skeletal system was examined for the presence of any deformity and signs of vitamin D deficiency such

as craniotabes, cranial bossing, persistent open anterior fontanelle, costo-chondral bending and epiphyseal widening. Thyroid gland was examined to find out any abnormality.

Thorough systemic examination was made to detect any abnormality in cardiovascular, digestive, respiratory and central nervous systems.

ANTHROPOMETRIC MEASUREMENTS :

Weight

Weight was recorded nearest to 0.1 kg by using adult type weighing machine. For children who could not stand, Infant Weighing scale was used, capable of measuring weight to the nearest 0.05 kg. Same machines were used for subsequent follow up, to minimise the error.

Length/Height

Recumbent length was measured by an Infantometer and standing height was taken by a locally fabricated Stadiometer. These measurements were recorded nearest to 0.1 cm.

Mid-Arm Circumference

Circumference of left upper arm at the point midway between the tip of the acromion process of scapula and Olecranon process of ulna, was measured, while arm was hanging freely, to the nearest 0.1 cm. A flexible steel tape was used to record this.

Laboratory investigations viz. haemoglobin, leucocyte count (total and differential), total serum

proteins, serum albumin, urine and stool examinations
were carried out routinely in each and every case.
Radiological and other relevant investigations were
performed if necessary.

Blood was collected by venepuncture. Samples
of sera were harvested and stored frozen at -20° until
ready for assay, but never for more than 4 months.

I - DETERMINATION OF TOTAL HAEMOLYTIC COMPLEMENT (CH_{50})

LEVEL :

Total haemolytic complement was determined
by the technique of Mayer (1961).

Principle :

Measurement of total serum haemolytic comple-
ment is a useful screening test for the integrity of
complement system. The test is based on the ability
of sheep red cells, properly sensitised by rabbit
antibody to sheep erythrocytes, to lyse in the presence
of all 9 classical pathway components. Haemoglobin
released by such lysis can be measured spectrophoto-
metrically with great precision and related to the
percentage of cells lysed.

Amount of complement required to lyse 50%
SRBC constitutes one unit of CH_{50}. Complement titre
is defined as the number of CH_{50} units contained in
1 ml of serum.

47

Material :

1. Alsever's Solution

Glucose 24.6 gm, trisodium citrate (dihydrate) 9.6 gm and sodium chloride 5.04 gm were dissolved in 1200 ml distilled water. The pH of solution was adjusted to 6.1 with 10% citric acid. It was then sterilized by low pressure autoclaving and stored in refrigerator. This solution was prepared fresh every 4 weeks. One volume or more was used for each volume of whole blood.

2. Stock Veronal Buffered Saline (Stock VBS)

A concentrated (5 times) solution was prepared by dissolving sodium chloride 83.0 gm, sodium 5,5 diethyl barbiturate 10.19 gm in 1.5 litres of distilled water. The pH of solution was adjusted to 7.35 ± 0.05 with 1 N HCl and volume was made upto 2.0 litres. This solution was stored for 1 month at $4°C$.

3. Isotonic Gelatin Veronal Buffered Saline (GVBS)

One part of stock VBS was mixed with 4 parts of distilled water. Sufficient dry gelatin was added to give final gelatin concentration of 0.1%. Gelatin was dissolved by gently heating and mixing the solution.

One ml each of $0.3 M CaCl_2$ and $2 M Mg Cl_2$ were added to each 1 litre of GVBS. This solution was prepared fresh every week.

4. Antisheep Haemolysin (procured commercially).

5. Normal Human Serum (NHS).

This was used as a source of complement (procured from a healthy donor).

6. Sheep Red Blood Cells (SRBC).

These were procured from jugular vein of a healthy sheep with the help of a dry sterilized syringe.

7. Test Serum.

(This was collected from the patient under investigation.)

Procedure :

1. Preparation of Sheep Red Blood Cells (SRBC) Suspension.

SRBC were collected in Alsever's solution and used 3-5 days after collection and within 12 days of collection. SRBC were stored at 4°C.

On the day of the test sheep erythrocytes were washed thrice in GVBS. One volume of the packed cells was suspended in 10 volumes of buffer to give a slightly greater than 5% suspension. One ml of this suspension was lysed with exactly 14 ml of distilled water and the optical density (O.D.) was measured at 541 mn with distilled water as blank.

A lysate with O.D. of 0.7 was considered to contain 5% or 1×10^9 Cells/ml. From the O.D. of the sample tested and volume of the suspension (V1), final volume (VF) to which the suspension was

adjusted to make a standardized suspension, was
calculated according to the relationship :

$$VF = \frac{VI \times O.D.}{0.7}$$

2. Titration of Haemolysin

This was first performed so that the
complement titration was independent of the concen-
tration of haemolysin. 5.0 ml volumes of 5% SRBC were
treated with equal volumes of 1:50, 1:100, 1:200, 1:400
and 1:800 diluted haemolysin in GVBS for 15 minutes at
37°C. This suspension of sensitized SRBC was now
called EA.

6.5 ml volumes of 1:50, 1:100, 1:200 and
1:400 diluted normal human serum (NHS) were also
prepared and taken in tubes A to D in 5 sets.

In tubes E and F, 6.5 ml. each of GVBS and
distilled water were taken, respectively. Then 1 ml.
of E A 1:50 was poured in 1st set of tubes from A to
F. The same procedure was repeated for EA 1:100, 1:200,
1:400 and 1:800 for rest of 4 sets of tubes.

NHS (1:50) 6.5 ml A	NHS (1:100) 6.5 ml B	NHS (1:200) 6.5 ml C	NHS (1:400) 6.5 ml D	GVBS 6.5 ml E	Distilled water 6.5 ml F
EA (1:50)1ml					
EA (1:100)1ml					
EA (1:200)1ml					
EA (1:400)1ml					
EA (1:800)1ml					

After mixing the contents of tubes in each
set, these were incubated at 37°C for 60 minutes. The
tubes were then centrifuged and optical density (O.D.)
measured at 541 mm. Percentage of haemolysis was
calculated by the formula :

$$Haemolysis\ (\%) = \frac{O.D.\ Row\ A\ to\ D\ -\ O.D.\ Row\ E}{O.D.\ Row\ F} \times 100$$

Dilution factors regarding antiserum (used
in EA suspension) and normal human serum were read and
used for further titration. These dilutions were found
to be 1:100 and 1:50 respectively in the present
series.

3. Titration of Complement

5% suspension of SRBC was prepared. Equal
volume of 1:100 diluted haemolysin was added to SRBC
suspension. This mixture was then incubated at 37°C
for 15 minutes and was stored in a refrigerator till
use (1 to 2 hours usually).

Test serum was diluted to 1:50 in GVBS and
titration was set up as follows :

TUBE	1	2	3	4	5	6
GVBS ml	4.0	3.0	1.5	0.5	6.5	-
Distilled water ml	-	-	-	-	-	6.5
EA ml	1.0	1.0	1.0	1.0	1.0	1.0
Test serum (1:50) ml	2.5	3.5	5.0	6.0	-	-

After mixing the contents of tubes in each column, the test material was incubated at 37°C for 60 minutes. Then tubes were centrifuged and optical density of the supernatant in each tube was read at 541 mm with distilled water containing tube as a blank.

4. Calculations to Determine the Number of Units of

Total Haemolytic Complement per ml of Serum (CH_{50})

Haemolysis (Y) was calculated for each tube as follows :

$$Y = \frac{O.D. \text{ tube 1 to 4 } - O.D. \text{ tube 5}}{O.D. \text{ tube 6}}$$

A graph was plotted. The log of the amount of test serum added (log X) was plotted on the abscissa; log $\frac{Y}{1 - Y}$ was plotted on the ordinate. The antilog of X where straight line crossed 0 (zero) on the ordinate gave the volume of test serum needed for 50% lysis.

CH_{50}U/ml of undiluted serum was calculated as follows:

$$CH_{50}\text{U/ml} = \frac{\text{dilution of serum}}{\text{volume required for 50\% lysis}}$$

II- DETERMINATION OF ALTERNATIVE PATHWAY ACTIVITY(AP_{50}):

Alternative pathway activity was assessed by the technique described by Platts-Mills and Ishizaka (1974).

Principle :

Unsensitized rabbit erythrocytes (RRBC) activate the alternative pathway of complement in

normal human serum. Hence the lysis of RRBC is used to assess the functional activity of the alternative pathway components.

Material :

1.   Alsever's Solution.

2.   Stock Veronal Buffered Saline (Stock VBS)

3.   Isotonic Gelatin Veronal Buffered Saline (GVBS)

For alternative pathway activity, only 1 ml of 2 M $MgCl_2$ was added to each 1 litre GVBS. This was prepared fresh every week.

4.   Stock EDTA

Disodium ethylene diamine tetra acetate 37.2 gm was dissolved in 800 ml distilled water. The pH was adjusted to 7.68 ± 0.05 with freshly prepared 2 M NaOH and volume was made to 1 litre. This was stored for 3 weeks at 4°C.

5.   GVBS - EDTA

Nine parts of isotonic GVBS (without $CaCl_2$ and $MgCl_2$) was mixed with 1 part of stock EDTA. This was prepared fresh every week.

6.   Rabbit Red Blood Cells. (These were procured by giving a cut on the ear margin of a healthy rabbit and blood collected under sterile conditions).

7.   Test Serum (This was collected from the patient).

Procedure :

RRBC, collected in Alsever's solution, were used from day 0 - 15 of collection. These were kept in the refrigerator after collection.

On the day of the test a 2.5% suspension of
RRBC in buffer was prepared in the same way as SRBC in
the determination of CH_{50}.

Test serum was diluted to 1:45 in GVBS and
titration was set up as follows :

TUBE		1	2	3	4	5	6
GVBS	ml	0.40	0.30	0.15	0.05	0.65	-
Distilled water	ml	-	-	-	-	-	0.65
RRBC	ml	0.10	0.10	0.10	0.10	0.10	0.10
Test serum (1:45)	ml	0.25	0.35	0.50	0.60	-	-

After mixing the tubes in each column, test
material was incubated at 37°C for 30 minutes. Reaction
was stopped by adding 3 ml of GVBS - EDTA. Tubes were
then centrifuged and optical density (O.D.) of the
supernatant was measured at 413 mμ.

Calculation was done exactly in the same way
as for CH_{50} and expressed as AP_{50} U/ml.

III- DETERMINATION OF C3 CONCENTRATION :

Serum C3 levels were estimated by single
radial immunodiffusion technique as described by
Mancini et al (1965), with suitable modifications
according to Fahey and McKelvey (1965).

Principle :

Antigen diffuses radially from the point of application into an antibody containing gel and a circular precipitate (ring) is formed at the zone of equivalence. Keeping antibody concentration and gel thickness constant, the area covered by precipitation ring is proportional to the concentration of antigen.

Material :

1. Stock Barbitone Buffer (0.12 N, pH 8.6)

Sodium barbitone 20.6 gm and barbitone 4.0 gm were dissolved in distilled water to a final volume of 1 litre with pH 8.6. One gm sodium azide was added per litre as a preservative.

2. Working Barbitone Buffer (0.06 N)

Stock was diluted to 1 : 2.

3. Monospecific Antiserm Against C3 (anti-C3)

(This was procured commercially).

4. Standard Normal Human Serum with Known Amount of C3

(This was procured commercially).

5. Unknown Test Serum

(This was obtained from the patient).

6. Glass slides of size 7.5 x 5.0 cm, gel punch, moist chamber, immunomensure.

Procedure :

Two percent Agar gel was prepared in working barbitone buffer. 2.5 ml volume of melted agar in a test tube was kept in a water bath maintained at 45°C.

2.5 ml of anti-C3 diluted 1:8 in buffer was added to the tube containing 2.5 ml agar and mixed thoroughly and poured over a clean glass slide kept on a horizontal table. Air bubbles, whenever present, were removed with a hot wire loop. Gel was allowed to set for 10-15 minutes.

Fifteen evenly spaced wells, of 3 mm size each, were cut, with the help of a punch using a predesigned template.

Measured volume (5 ul each) of various dilutions of standard serum and appropriately diluted test sera, were run into the wells. The plate was then incubated in a moist chamber at room temperature and diffusion was allowed for 20 hours. Finally the diameter (d) of precipitin rings was measured by immunomeasure and d^2 (diameter square) was obtained in mm (Fig.: 4).

A standard curve was drawn by plotting d^2 vs known concentrations of standard serum. Concentrations of unknowns (with known diameter) were obtained from the curve. Final results were obtained after multiplying the readings with the dilution factor and expressed in mg/dl.

***
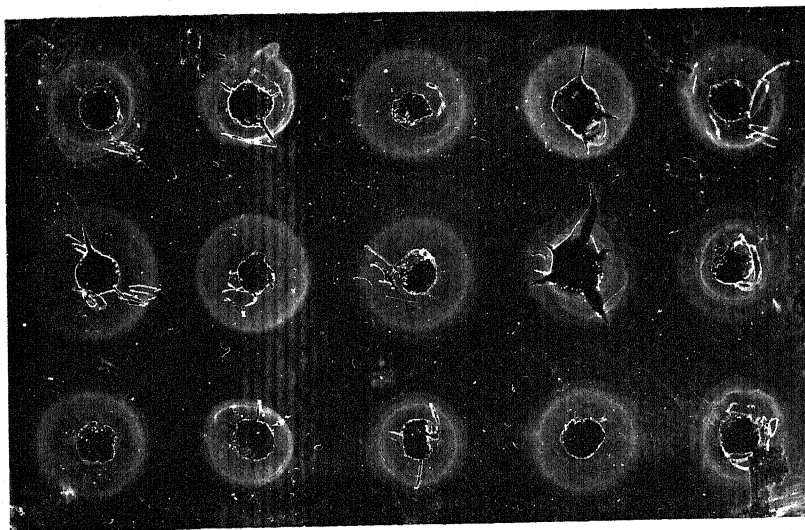
Fig.4 : Showing Single Radial
        Immunodiffusion.

OBSERVATIONS

A study to assess the functional activity of complement system in protein-calorie malnutrition(PCM) was carried out in 32 pre-school children(1-5 years age) at M.L.B. Medical College, Jhansi, between May 1981 and March 1983. Besides complement activity, various anthropometric measurements, serum albumin and blood haemoglobin values were noted in each case. A control group, comprising 12 age matched well nourished children, was also studied for comparative evaluation of complement activity, anthropometric measurements, serum albumin and haemoglobin values.

Age distribution of control and PCM cases is shown in Table I.

TABLE I

Age distribution of control and PCM cases.

Clinical group	Age distribution (months)				Total
	12-24	24-36	36-48	48-60	
Control	5	4	1	2	12
PCM	18	9	4	1	32
Total	23	13	5	3	44

Cases of PCM, 32 in number were further divided into 3 clinical groups according to McLaren classification. Based on this classification, 19 cases

cases had a definite history of either pertusis,
measles or worm infestation.

Family history of tuberculosis was elicited
in 6 cases.

All the cases, at the time of admission, were
receiving diet, grossly deficient in calories as well as
proteins.

None of the 12 control cases had a history of
infections in the immediate past and they were not suffer-
ing from any demonstrable illness at the time of inclusion
in this study.

I - INITIAL CONTACT :

1- Anthropometric Values :

Anthropometric profile of control and PCM cases
at the initial contact is depicted in Table III.

As is evident from Table, mean anthropometric
values of weight, length (height) and mid-arm circumfer-
ence in children suffering from PCM were appreciably less
than in controls. However different groups of PCM, as
per McLaren classification, did not reveal clear differ-
ences in the anthropometric measurements.

2- Serum Albumin, Haemoglobin Values and Complement
   Activity in Two Study Groups :

Mean serum albumin deemed to be significantly
depressed in PCM (2.12 ± 0.80 gm/dl) as compared to
controls (P < 0.001). Similarly mean haemoglobin value

Table III

Anthropometric profile in control and PCM cases

Clinical group	No.of cases	Age(months) Mean ± S D	Weight(kg) Mean ± S D	Length or Height(cm) Mean ± S D	Mid-arm circumference (cm) Mean ± SD	Weight expressed as % of 50th percentile of Harvard Standard Mean ± SD	Length(height) expressed as % of 50th percentile of Harvard Standard Mean ± SD	Mid-arm circumference expressed as % of normal Standard Mean ± SD
Control	15	38.13 ± 16.56	13.99 ± 3.98	97.71 ± 11.55	13.48 ± 0.76	99.63 ± 17.44	95.17 ± 2.82	94.64 ± 4.20
PCM	58	27.13 ± 18.37	6.73 ± 1.51	78.95 ± 9.67	9.98 ± 1.55	58.91 ± 8.96	90.58 ± 5.27	64.41 ± 9.05
Marasmus	19	28.94 ± 18.06	6.67 ± 1.61	74.79 ± 6.88	10.08 ± 1.47	51.88 ± 5.07	88.36 ± 5.55	68.15 ± 9.07
Marasmic kwashiorkor	10	28.40 ± 18.00	6.58 ± 1.55	78.67 ± 7.55	9.74 ± 1.57	54.37 ± 5.40	94.95 ± 5.35	60.58 ± 9.90
Kwashiorkor	9	30.00 ± 11.70	6.97 ± 1.70	71.50 ± 7.40	9.90 ± 2.28	55.03 ± 3.42	80.33 ± 5.52	69.59 ± 12.90

$(7.88 \pm 1.68 \text{ gm/dl})$ was significantly lower in PCM as compared to controls $(P < 0.001)$, as shown in Table IV.

Complement activity was evaluated by three important parameters viz. total haemolytic complement $(CH_{50} U/ml)$, alternative pathway activity $(AP_{50} U/ml)$ and C3 concentration (mg/dl).

Table IV shows that at the time of initial contact mean CH_{50} value in PCM group was 8.89 ± 3.48 U/ml and mean AP_{50} value was 64.66 ± 23.07 U/ml. A comparison of mean CH_{50} and AP_{50} values between control and PCM group revealed that the differences were not statistically significant $(P > 0.08)$. However, mean serum C3 concentration $(60.89 \pm 33.25 \text{ mg/dl})$ in the PCM group was found to be significantly lower than in the control group $(P < 0.001)$.

3- Serum Albumin, Haemoglobin, CH_{50}, AP_{50} and C3 Values

in Various Groups of PCM :

Mean serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in various groups of PCM as per McLaren classification viz. marasmus, marasmic-kwashiorkor and kwashiorkor are depicted in Table V.

Table IV

Serum albumin, hemoglobin, CH_{50}, AP_{50} and C3 values in two study groups.

Clinical Group	No. of cases	Serum albumin (gm/dl) Mean ± S D	Hemoglobin (gm/dl) Mean ± S D	CH_{50} (u/dl) Mean ± S D	AP_{50} (u/dl) Mean ± S D	C3 (mg/dl) Mean ± S D
Control	12	4.05 ± 0.37	12.57 ± 1.04	7.16 ± 1.93	64.70 ± 10.11	125.93 ± 23.98
PCM	38	3.12 ± 0.50	7.98 ± 1.62	5.99 ± 2.45	61.66 ± 9.45	60.89 ± 23.98
P value		< 0.001	< 0.001	> 0.05	> 0.05	< 0.001

Table V

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in controls and various groups of PCM

	Control Mean ± S.D	Kwashiorkor Mean ± S.D	Significance vs control	Marasmic-kwashiorkor Mean ± S.D	Significance vs control	Marasmus Mean ± S.D	Significance vs control
Serum albumin (g/dl)	4.05 ± 0.37	3.49 ± 0.56	< 0.001	3.72 ± 0.41	< 0.001	3.05 ± 1.43	< 0.001
Haemoglobin (g/dl)	13.57 ± 1.05	9.64 ± 1.14	< 0.001	6.96 ± 1.60	< 0.001	9.60 ± 0.93	< 0.001
CH_{50} (U/ml)	7.16 ± 1.93	5.81 ± 3.60	> 0.05	5.77 ± 3.70	> 0.05	5.98 ± 3.96	> 0.05
AP_{50} (U/ml)	64.70 ± 10.11	63.88 ± 24.91	> 0.05	63.88 ± 22.85	> 0.05	61.40 ± 9.90	> 0.05
C3 (mg/dl)	135.08 ± 23.98	63.16 ± 23.98	< 0.001	63.30 ± 29.68	< 0.001	38.67 ± 3.22	< 0.001

Serum Albumin :

Mean serum albumin values (gm/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were 3.49 ± 0.53, 2.72 ± 0.41 and 2.09 ± 1.62 at the initial contact (Fig.5). These values were significantly lower as compared to controls (P < 0.01 in marasmus and < 0.001 in rest two groups). Mean serum albumin value was lowest in cases suffering from kwashiorkor, when first seen.

Haemoglobin :

Mean haemoglobin values (gm/dl) were found to be 8.62 ± 1.14, 6.96 ± 1.60 and 5.60 ± 0.53 in marasmus, marasmic-kwashiorkor and kwashiorkor respectively. These values were found to be significantly lower as compared to controls (P < 0.001). Mean haemoglobin percentage was the lowest in kwashiorkor cases as compared to other two groups.

CH_{50} :

Mean CH_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor groups at the time of first contact were 5.51 ± 5.68, 5.77 ± 2.70 and 5.48 ± 5.96 respectively (Fig.6). A comparison of these mean values with those in control subjects revealed that all 3 groups each had lower but not statistically significant mean values, as compared to control group ($p > 0.05$). Also the group differences of mean CH_{50} values were not appreciably different.

SERUM ALBUMIN CONCENTRATION IN CONTROL AND PCM
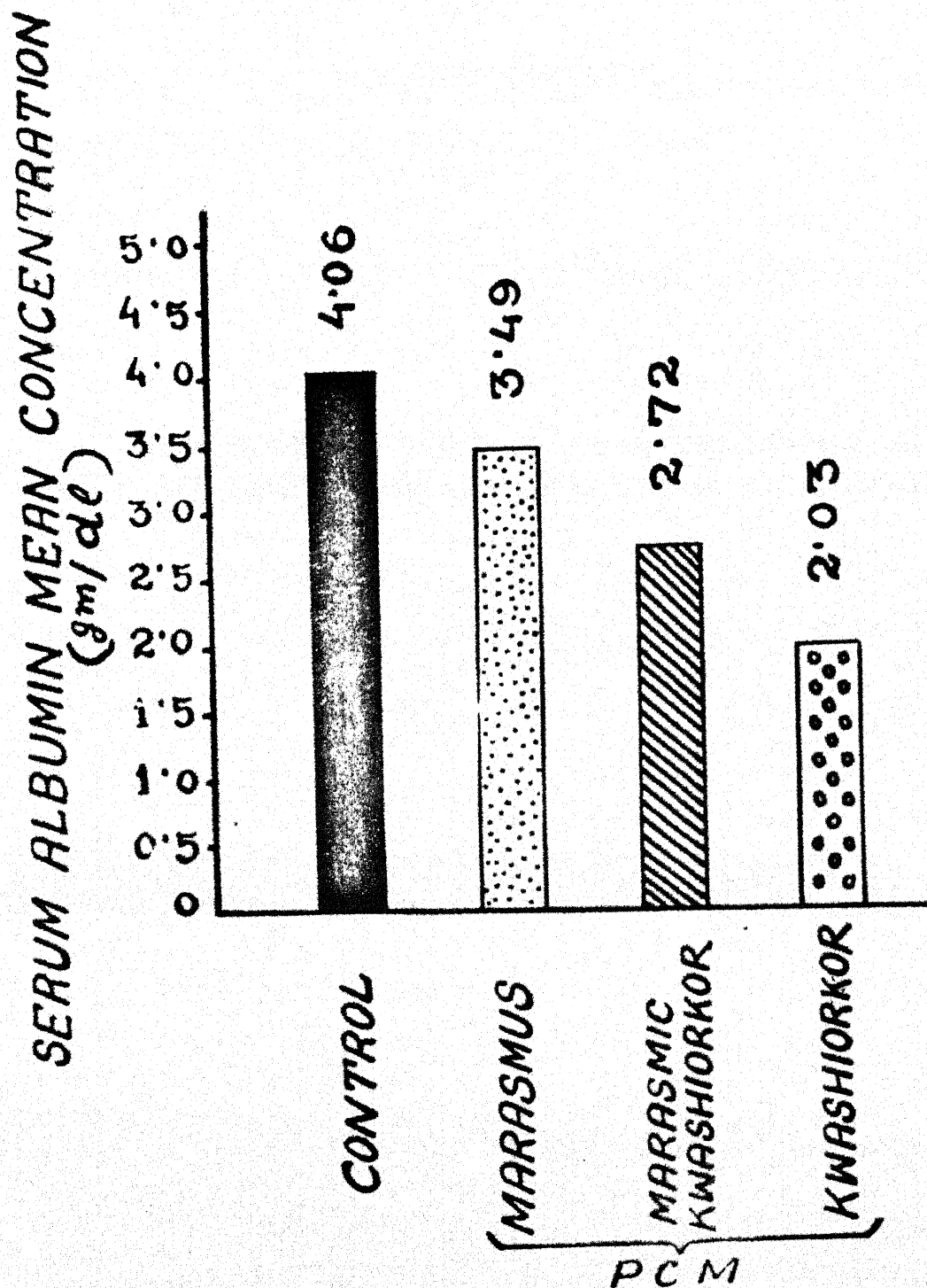
FIG. No. 5.

TOTAL HAEMOLYTIC COMPLEMENT (CH50) ACTIVITY IN CONTROL AND PCM.
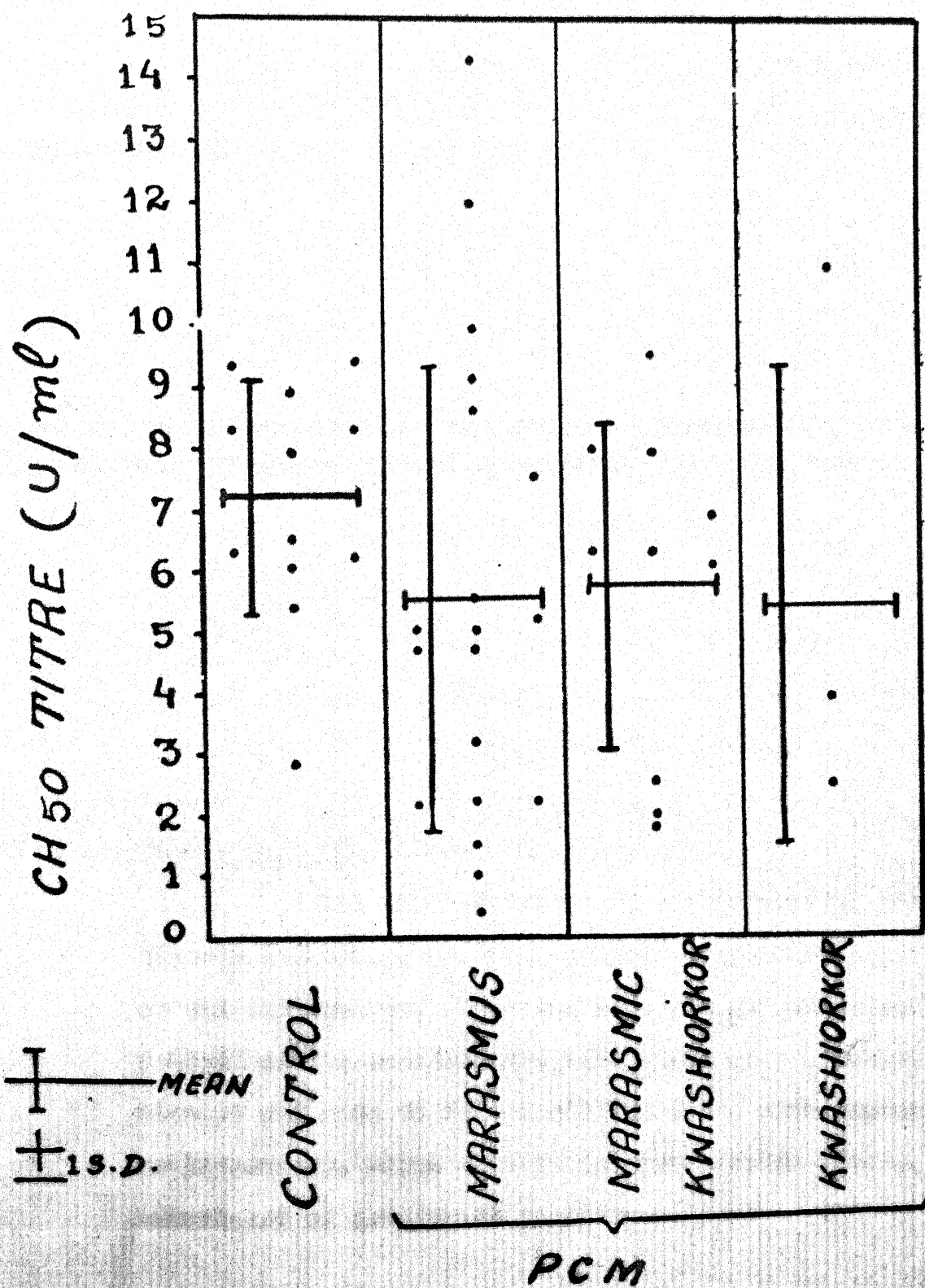
FIG. No. 6.

AP_{50}:

Mean AP_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor, on initial contact, were 63.02 ± 24.91, 62.22 ± 22.83 and 51.40 ± 3.90 respectively (Fig.17). It is evident that mean values in three groups of PCM were lower as compared to controls. However, mean AP_{50} value in kwashiorkor group was maximally depressed and statistically significant ($P < 0.05$).

Complement C3 :

Mean C3 values (mg/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were found to be 63.16 ± 23.98, 62.30 ± 22.88 and 39.67 ± 2.31 respectively (Fig. 8). These values were significantly lower as compared to control cases ($P < 0.001$). However, the mean values in 3 groups were not appreciably different from each other.

II- FOLLOW UP :

After the assessment of anthropometry, serum albumin and haemoglobin values, and complement activity on initial contact, PCM cases were put on nutritional rehabilitation schedule and infections were treated. An attempt was made to follow all PCM cases with repeat anthropometry, serum albumin and haemoglobin levels, and assessment of complement activity.

ALTERNATIVE PATHWAY ACTIVITY (AP$_{50}$) IN CONTROL AND PCM
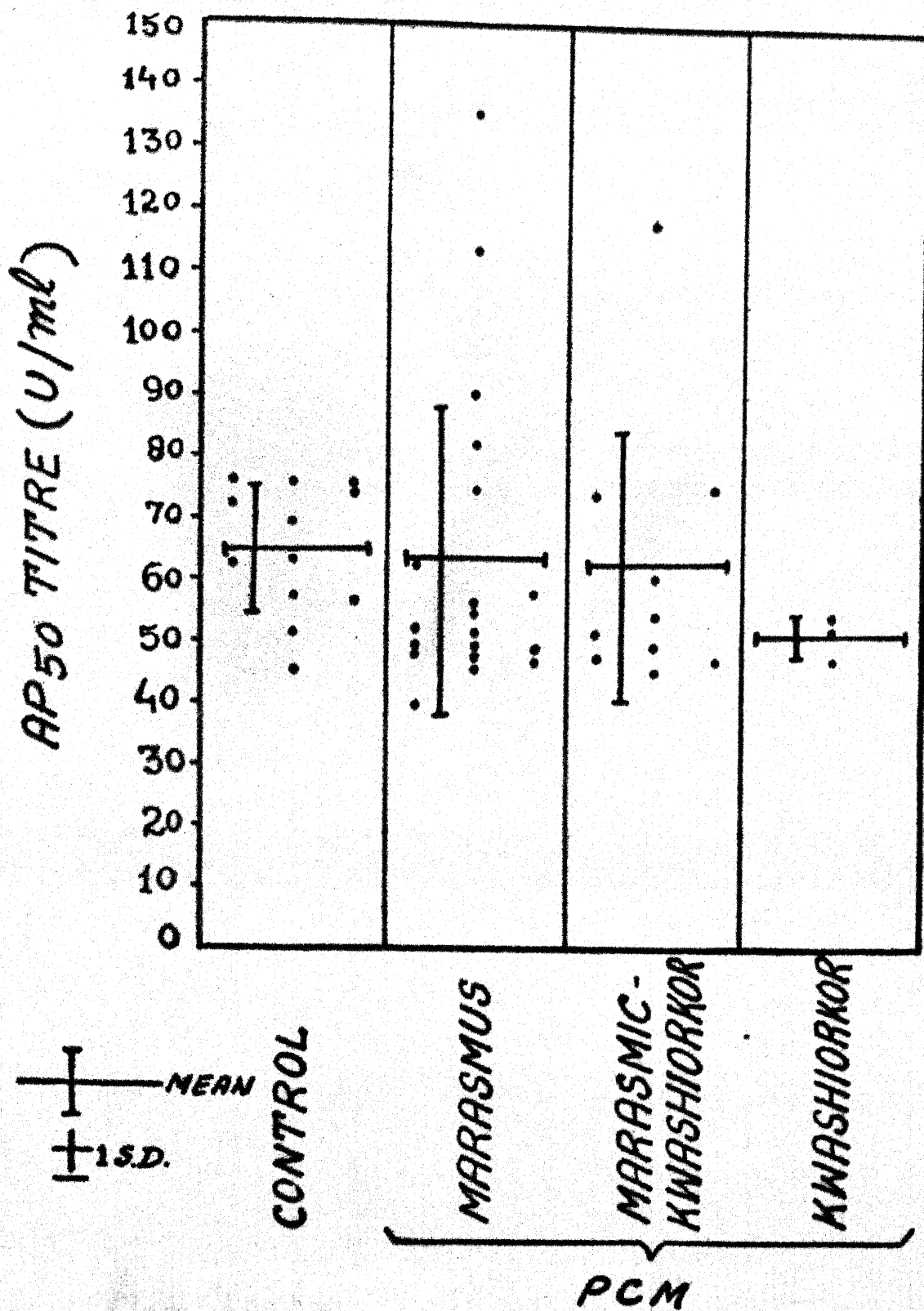
FIG. No. 7.

COMPLEMENT C3 CONCENTRATION IN CONTROL AND PCM

FIG.No.8.
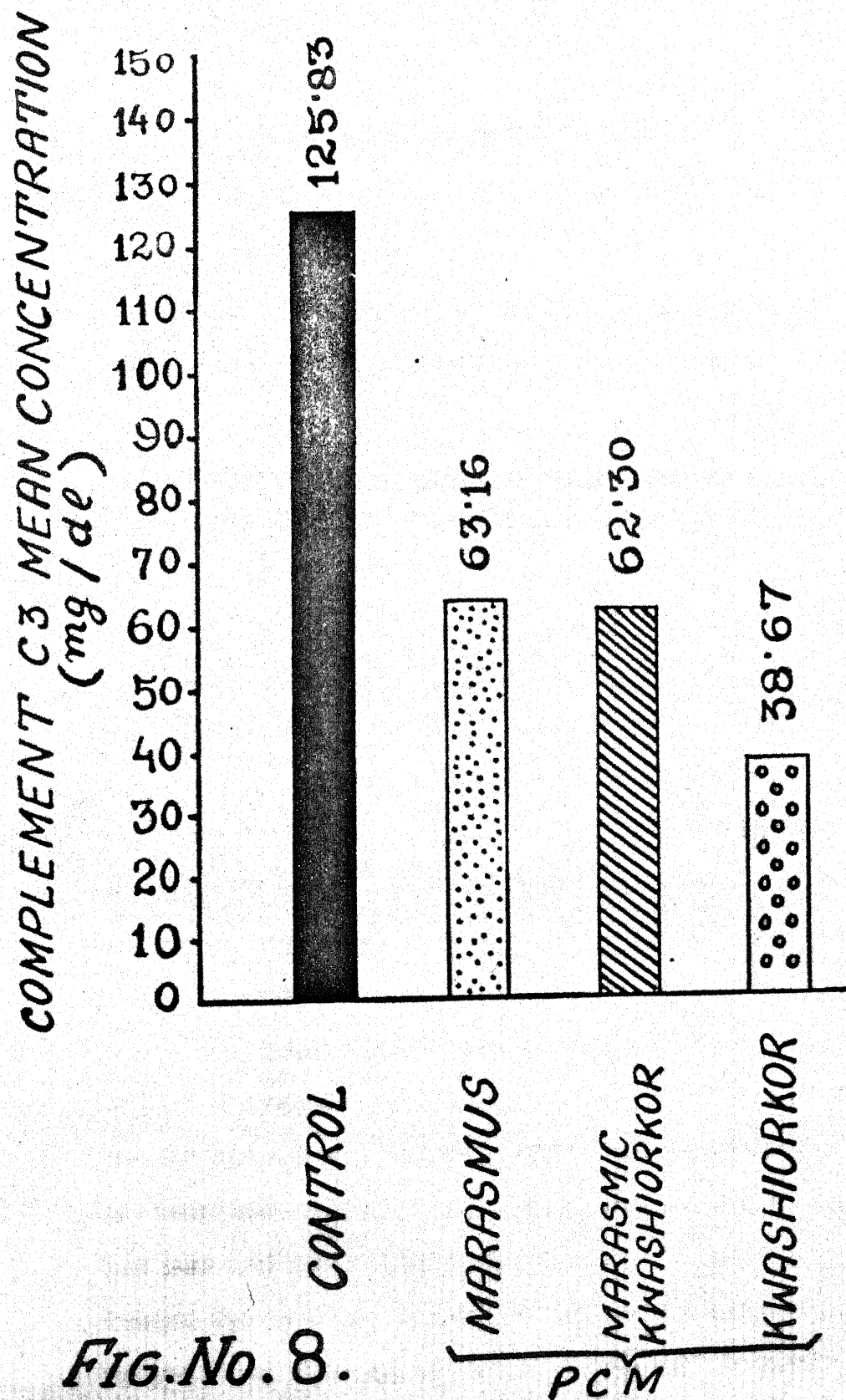
In first follow up after an interval of 2 weeks 17 cases could be taken; in 2nd follow up, after 4-7 weeks, 7 cases turned up and 3rd follow up could be done after 10-12 weeks in only 2 cases.

1. Anthropometric Profile :

Marasmus Group :

Anthropometric profile in the follow up groups of marasmus is shown in Table VI.

Table VI

Anthropometric profile in follow up groups of marasmus.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	19	6.67 ± 1.24	74.79 ± 6.80	10.03 ± 1.47
1st follow up	9	7.91 ± 1.05	75.39 ± 5.64	11.16 ± 1.31
2nd follow up	4	8.04 ± 1.66	74.18 ± 6.60	12.50 ± 1.31
3rd follow up	1	11.50	83.50	14.40

Table VI shows that improvement in weight and mid-arm circumference were sustained during follow up. Mean weight increased by 1.24 kg and 1.37 kg in the first and second follow up, respectively. Arm circumference showed an increase of 1.13 cm and 2.47 cm in mean values, after 1st and 2nd follow up respectively. A single case, who turned up on three occasions showed a consistent rise in weight from 8.00 kg at initial contact to 9.00 kg, 9.70 kg and 11.50 kg on 2nd, 3rd and 4th contact respectively.

Similarly arm circumference values in the same case improved from 11.50 cm at the initial contact to 12.40 cm, 14.20 cm and 14.40 cm at subsequent dates during follow up. However, improvement in mean length (height) did not seem to be constant during follow up period.

Marasmic-kwashiorkor Group :

Anthropometric profile in the follow up groups of marasmic-kwashiorkor is depicted in Table VII.

Table VII

Anthropometric profile in follow up groups of marasmic-kwashiorkor.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	10	6.89 ± 1.86	73.67 ± 7.07	9.74 ± 1.57
1st follow up	7	7.21 ± 1.08	74.24 ± 7.68	9.99 ± 0.77
2nd follow up	3	6.90 ± 0.40	68.87 ± 6.70	10.97 ± 0.80
3rd follow up	1	11.00	78.00	13.00

In marasmic-kwashiorkor group, gain in mean weight was 0.62 kg and 0.21 kg during 1st and 2nd follow up dates. However, one individual, who turned up on all three occasions, showed a weight increase of 1.15 kg, 1.55 kg and 5.25 kg respectively. Again in arm circumference values, there was a sustained improvement during 3 follow ups viz. 0.25 cm and 1.23 cm and an individual case

who had three repeat measurements showed an increase of
0.80 cm, 1.80 cm and 2.30 cm during 1st, 2nd and 3rd
follow up respectively. As with marasmus, no constant
improvement was noticed in length (height) measurement
during the follow up.

Kwashiorkor Group :

Anthropometric profile in the follow up group
of kwashiorkor is shown in Table VIII.

Table VIII

Anthropometric profile in follow up group of kwashiorkor.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	3	6.97 ± 1.70	71.80 ± 7.86	9.93 ± 2.20
1st follow up	1	7.80	67.00	12.00

In this group, only one case turned up at the
first follow up. After a period of 2 weeks treatment on
prescribed diet, weight showed an increase from initial
6.90 kg to 7.00 kg, subsequently. Length increased from
66.00 cm initially, to 67.00 cm after 2 weeks. Arm circum-
ference increased from 11.20 cm to 12.00 cm after 2 weeks
of nutritional rehabilitation.

2. Serum Albumin, Haemoglobin, CH_{50}, AP_{50} and C3 Values :

Marasmus Group :

Mean values of serum albumin, haemoglobin, CH_{50},
AP_{50} and C3 are shown in Table IX.

Table II

Serum albumin, hemoglobin, CH_{50}, AP_{50} and C3 values in calves in follow up groups of newborns.

Clinical group	No. of calves	Serum albumin (gm/dl) Mean ± S D	Hemoglobin (gm/dl) Mean ± S D	CH_{50} (U/ml) Mean ± S D	AP_{50} (U/ml) Mean ± S D	C3 (mg/dl) Mean ± S D
Initial sample	19	2.40 ± 0.45	9.65 ± 1.44	8.86 ± 3.08	63.88 ± 24.91	65.16 ± 22.88
1st follow up	9	2.91 ± 0.52	9.76 ± 1.02	10.24 ± 2.83	64.45 ± 8.65	135.33 ± 42.25
2nd follow up	4	2.97 ± 0.75	10.70 ± 0.89	8.61 ± 3.93	63.16 ± 10.51	168.00 ± 34.99
3rd follow up	1	9.90	11.00	7.92	95.66	159.00

Serum Albumin :

There was a sustained increase in mean serum albumin levels during first and second follow up periods, being 0.42 gm/dl and 0.48 gm/dl respectively. In a single case who turned up for 3rd follow up, 0.50 gm/dl rise in mean serum albumin level was observed. Thus a sustained increase in albumin levels was observed throughout the follow up, in worseuus cases.

Haemoglobin :

Like serum albumin levels, mean haemoglobin values increased from 8.62 gm/dl initially to 9.76 gm/dl and 10.70 gm/dl in 1st and 2nd follow up, respectively. At the 3rd follow up, Hb level increased from initial 7.80 gm/dl to 11.00 gm/dl in a single case observed.

CH_{50} :

From Table IX, showing the effect of nutritional repair on CH_{50} levels, it is evident that CH_{50} attained maximum mean level (10.84 U/ml) after 1st follow up as compared to initial mean of 8.81 U/ml; a rise of 4.73 U/ml in mean value. Although, in 2nd follow up, the mean level decreased (8.61 U/ml) yet it remained higher than the initial with a difference of 3.10 U/ml. A case who turned up for 3rd follow up showed that CH_{50} value decreased from 11.99 U/ml at initial contact to 7.98 U/ml at the last follow up.

AP_{50} :

On evaluation of AP_{50} values in follow up groups, it is clear that there was no constant difference in repeat values as compared to initial one.

Complement C3 :

There was a rise in mean serum C3 concentration after the 1st and 2nd follow up being 72.17 mg/dl and 100.04 mg/dl from the initial. However, in a single case, followed 3rd time, a rise of only 26 mg/dl in C3 value was observed from initial contact to last follow up (Fig. 19).

Marasmic-Kwashiorkor Group :

Mean values of serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 are depicted in Table X.

Serum Albumin :

Table X reveals that between the initial contact and 1st follow up there was rise of 1.15 gm/dl in mean serum albumin level as compared to the initial sample. However, there was no further rise in the mean albumin level at second follow up. Maximum increase of albumin value i.e. 2.10 gm/dl was observed in a single case after 3rd follow up.

Haemoglobin :

Mean Hb values showed a sustained increase during the 1st and 2nd follow up assessment, being 1.95 gm/dl and 3.64 gm/dl respectively. However, after 3rd follow up in a single case, there was a rise of only 1.30 gm/dl in haemoglobin from the initial value.

COMPLEMENT C3 CONCENTRATION IN INITIAL AND FOLLOWUP GROUPS OF MARASMUS
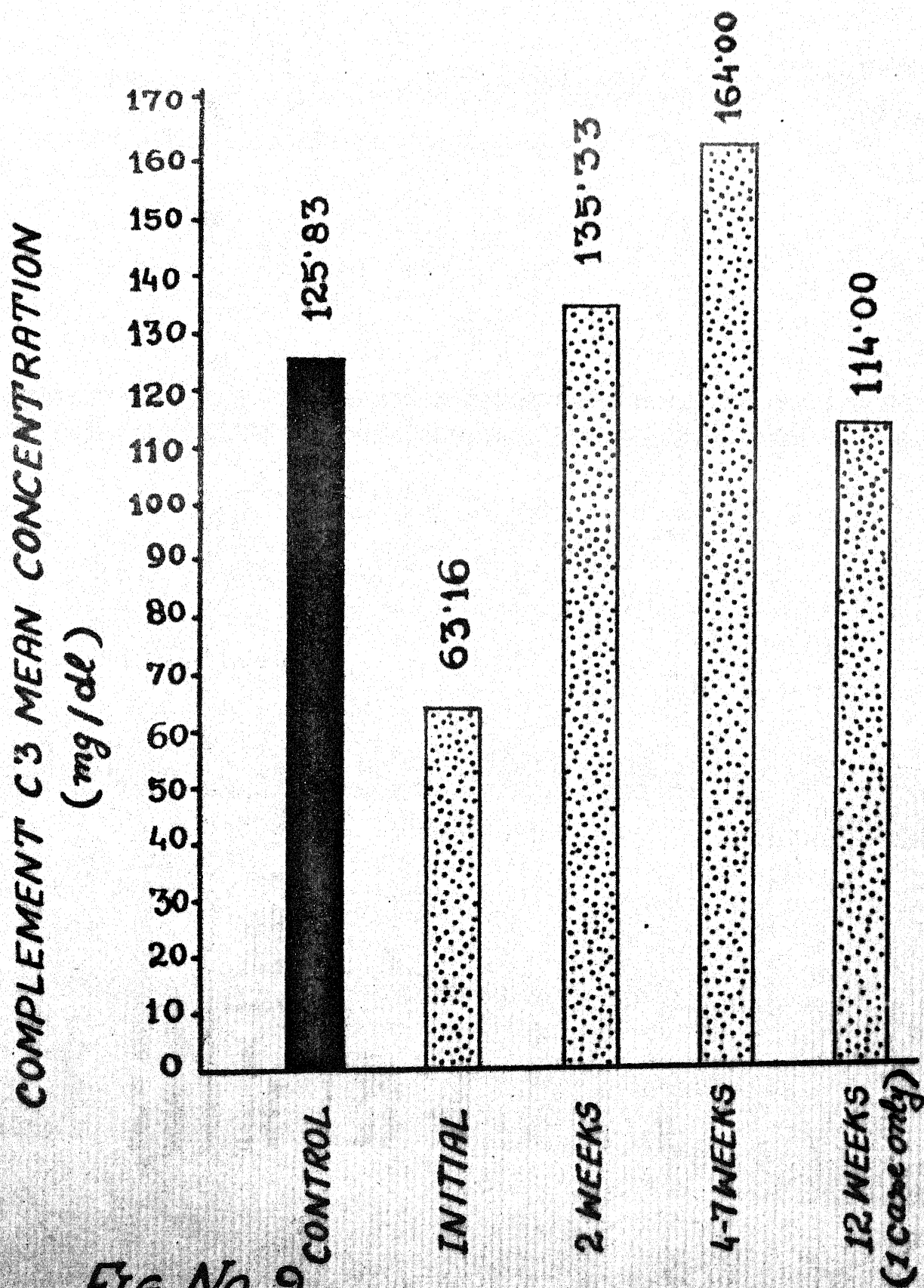
COMPLEMENT C3 MEAN CONCENTRATION (mg / dl)

- CONTROL: 125·83
- INITIAL: 63·16
- 2 WEEKS: 135·33
- 4-7 WEEKS: 164·00
- 12 WEEKS (1 case only): 114·00

FIG. No. 9.

Table X

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in follow up groups of rheumato-heptatitor.

Clinical group	No. of cases	Serum albumin (gm/dl) Mean ± S D	Haemoglobin (gm/dl) Mean ± S D	CH_{50}(U/ml) Mean ± SD	AP_{50}(U/ml) Mean ± S D	C3 (mg/dl) Mean ± S D
Initial sample	10	3.72 ± 0.41	6.96 ± 1.60	5.77 ± 2.70	68.82 ± 23.83	68.90 ± 22.38
1st Follow up	7	3.57 ± 0.38	8.89 ± 0.98	9.23 ± 3.84	59.63 ± 11.95	123.14 ± 44.16
2nd Follow up	3	3.92 ± 0.15	10.07 ± 0.96	7.20 ± 4.09	61.03 ± 15.96	112.00 ± 45.98
3rd Follow up	1	5.10	10.60	6.39	65.13	143.00

CH_{50} :

It was observed that mean CH_{50} level raised by 3.46 U/ml after 1st follow up. Although mean CH_{50} decreased in 2nd follow up as compared to the value in 1st follow up, yet it remained higher by 1.43 U/ml than what it was at the initial contact. Maximum rise of 3.78 U/ml, was observed in a single case, between the initial contact and 3rd follow up.

AP_{50} :

As with marasmus group, there was no consistent pattern of rise in AP_{50} values during the follow up.

Complement C3 :

On evaluation of mean C3 levels, a rise of 60.84 mg/dl was observed during 1st follow up. Although after 2nd follow up mean value decreased, it still persisted at higher level than it was at the time of initial contact, the difference being of 49.70 mg/dl. Maximum increase in C3 concentration viz. 111.00 mg/dl was noticed in a single case between the initial contact and 3rd follow up (Fig.:10).

Kwashiorkor Group :

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values are shown in Table XI.

In this group only one case could be followed up, after 2 weeks from the initial contact.

Serum Albumin :

Serum albumin value increased from 1.50 gm/dl initially, to 2.90 gm/dl after 2 weeks of nutritional therapy.

COMPLEMENT C3 CONCENTRATION IN INITIAL, AND FOLLOWUP GROUPS OF MARASMIC KWASHIORKOR

FIG. No. 10.

Table II

Serum albumin, hemoglobin, GPT, AP and CB levels in sera in group of tumbletor.

Clinical group	No. of cases	Serum albumin (gm/dl) Mean ± S D	Hemoglobin (gm/dl) Mean ± S D	GPT (U/dl) Mean ± S D	AP (U/dl) Mean ± S D	CB (mg/dl) Mean ± S D
Initial sample	8	3.08±1.48	3.60±0.58	3.48±3.96	31.40±3.90	38.67±3.91
1st follow up	1	3.90	7.00	11.96	45.00	93.00

Haemoglobin :

As with serum albumin, there was a rise of 2.00 gm/dl in haemoglobin value after 2 weeks follow up.

CH$_{50}$:

CH$_{50}$ value increased by 2.88 U/ml after 2 weeks.

AP$_{50}$:

In contrast to other parameters, AP$_{50}$ value decreased from 47.12 U/ml to 45.00 U/ml after a 2 weeks follow up.

Complement C3 :

On evaluation of C3 concentration, a significant rise of 82.00 mg/dl was observed in 2 weeks time (Fig. 11).

Correlation of Age with Complement Activity :

Table XII shows the mean values of CH$_{50}$, AP$_{50}$ and C3 complement in different age groups.

Table XII

Correlation of age with complement activity.

1 Age group (months)	No. of cases	2 CH$_{50}$ (U/ml) Mean ± S D	3 AP$_{50}$ (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
12 - 24	33	5.65 ± 3.11	63.01 ± 22.68	61.13 ± 37.51
24 - 36	12	6.40 ± 3.28	68.99 ± 20.38	71.05 ± 40.63
36 - 48	5	5.22 ± 2.82	54.29 ± 10.71	71.80 ± 42.00
48 - 60	3	5.85 ± 5.29	65.63 ± 12.89	96.67 ± 12.01

$r_{1,2}$ = 0.162 (P > 0.05)

$r_{1,3}$ = -0.113 (P > 0.05)

$r_{1,4}$ = -0.002 (P > 0.05)

COMPLEMENT C3 CONCENTRATION IN INITIAL AND FOLLOWUP GROUPS OF KWASHIORKOR

COMPLEMENT C3 MEAN CONCENTRATION (mg./dl)

125·83 — CONTROL

38·67 — INITIAL

92·00 — 2 WEEKS (1 case only)
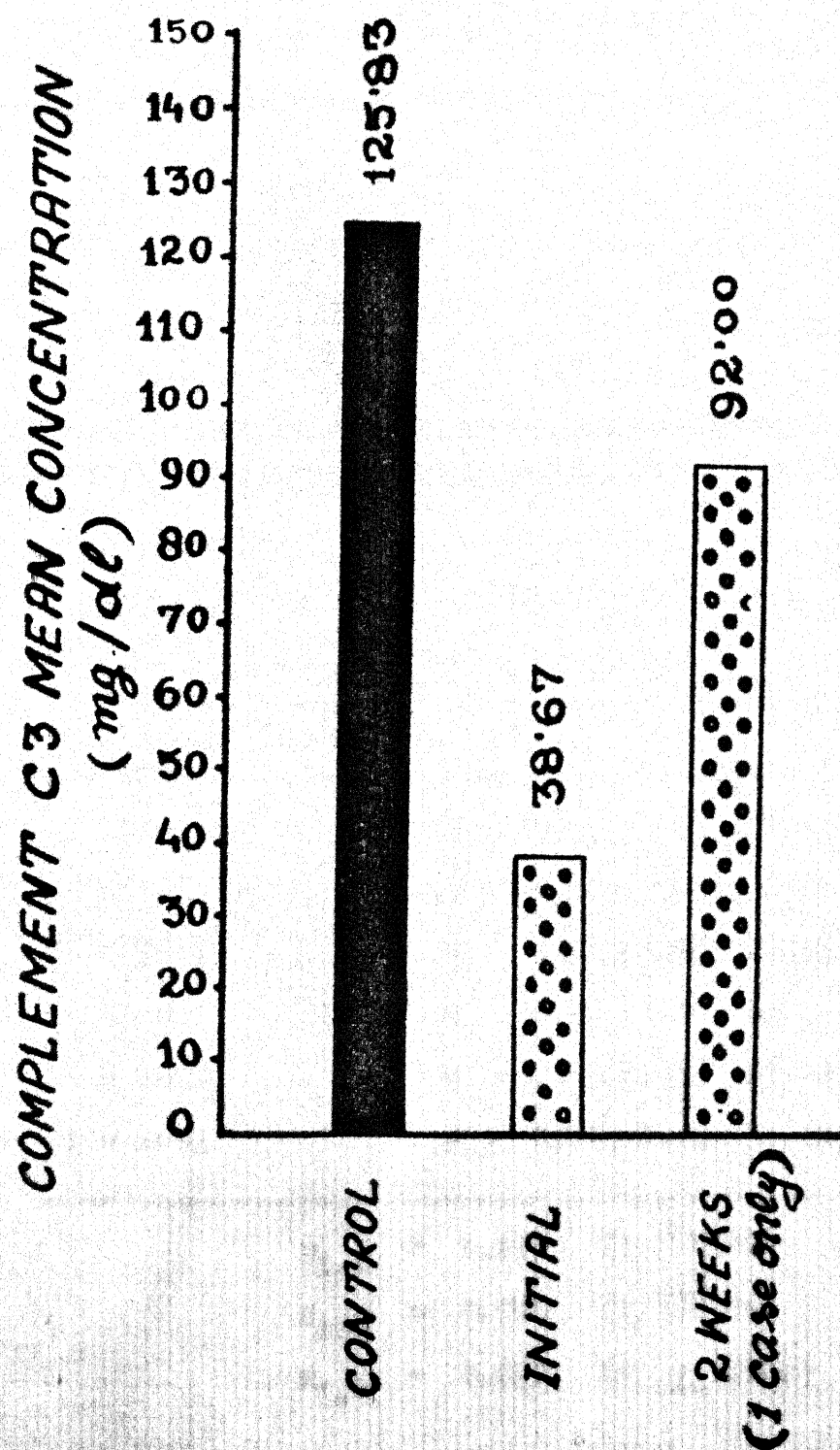
FIG.No. 11.

It is obvious from Table that no definite trend of rise or decline was seen in complement values in relation to age. Correlation coefficient (r) of age with CH_{50}, AP_{50} and C3 were observed to be 0.162, -0.118 and -0.002 respectively, all being non-significant (P > 0.05).

Correlation of Weight (expressed as % of 50th Percentile of Harvard Standard) with Complement Activity :

Table XIII shows the mean values of CH_{50}, AP_{50} and C3 complement in different percentage weight groups.

Table XIII
Correlation of weight (expressed as % of 50th percentile of Harvard Standard) with complement activity.

1 Weight expressed as % of 50th percentile of Harvard Standard	No.of cases	2 CH_{50}(U/ml) Mean ± S D	3 AP_{50}(U/ml) Mean ± S D	4 C3(mg/dl) Mean ± S D
⪕ 50%	13	4.97 ± 3.15	57.44 ± 20.43	66.13 ± 26.91
51 - 60 %	14	6.68 ± 3.92	61.76 ± 34.92	85.36 ± 21.37
61 - 70 %	8	3.98 ± 3.16	57.87 ± 22.05	62.33 ± 34.05
71 - 80 %	2	4.89 ± 0.19	53.01 ± 6.60	80.00 ± 8.49
81 - 90 %	5	7.68 ± 1.70	66.43 ± 9.02	124.20 ± 30.61
91 - 100%	7	6.78 ± 3.11	62.03 ± 10.64	126.29 ± 30.70

$$r_{1,2} = 0.193 \quad (P > 0.05)$$
$$r_{1,3} = 0.099 \quad (P > 0.05)$$
$$r_{1,4} = 0.605 \quad (P < 0.001)$$

It is clear from Table that no definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to weight percentage. Correlation coefficient (r) of weight percentage with CH_{50} and AP_{50} were observed to be 0.198 and 0.099 respectively, being non-significant ($P > 0.05$). However, a positive correlation (r = 0.685) was seen between weight percentage and C3 values, being statistically significant ($P < 0.001$).

Correlation of Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

Mean values of CH_{50}, AP_{50} and complement C3 in different groups of length (height) percentage are shown in Table XIV.

Table XIV

Correlation of length/height (expressed as % 50th percentile of Harvard Standard) with Complement activity.

1 Length/height expressed as % of 50th percentile of Harvard Standard.	No.of cases	2 CH_{50}(U/ml) Mean ± S D	3 AP_{50}(U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
70 - 80 %	7	6.96 ± 2.96	82.62 ± 12.09	65.97 ± 24.96
81 - 90 %	21	5.47 ± 2.69	56.11 ± 18.14	89.05 ± 24.17
91 - 100%	16	6.80 ± 2.46	72.84 ± 22.60	109.38 ± 37.07

$r_{1,2} = 0.076$ ($P > 0.05$)
$r_{1,3} = 0.222$ ($P > 0.05$)
$r_{1,4} = 0.636$ ($P < 0.001$)

It is evident from Table that correlation coefficient (r) of length percentage with CH_{50} was observed to be 0.076, being non-significant ($P > 0.05$). Although there was

a rising trend in AP_{50} values in relation to length percentage, correlation coefficient (r = 0.222) between them was non-significant (P>0.05). However, a positive correlation (r = 0.636) was observed length percentage and C3 values, being statistically significant (P<0.001).

Correlation of Mid-Arm Circumference with Complement Activity :

The mean values of CH_{50}, AP_{50} and complement C3 in different groups of mid-arm circumference are depicted in Table XV.

Table XV

Correlation of mid-arm circumference with complement activity.

1 Mid-arm circumference (cm)	No.of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
6 - 9	10	4.77±2.78	60.65±24.12	87.70±30.07
9 - 12	20	5.98±3.07	61.96±24.13	61.50±24.94
12 - 15	4	7.86±1.84	67.08±9.12	90.50±40.11
15 - 18	10	6.62±1.98	87.67±20.98	128.00±22.07

$$r_{1,2} = 0.266 \quad (P>0.05)$$
$$r_{1,3} = 0.242 \quad (P>0.05)$$
$$r_{1,4} = 0.721 \quad (P<0.001)$$

It is obvious from the Table that no definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation of mid-arm circumference. Correlation coefficients (r) of mid-arm circumference with CH_{50} and AP_{50} were found to be 0.266 and 0.242 respectively, being non-significant (P>0.05). However, there was definite rising

trend in C3 values in relation to arm circumference,
correlation coefficient (r) being 0.721 and significant
(p $<$ 0.001).

Correlation of Serum Albumin Concentration and Complement
Activity :

Table XVI shows the mean values of CH_{50}, AP_{50} and
complement C3 in different groups of serum albumin values.

Table XVI

Correlation of serum albumin concentration with complement
activity.

1	2	3	4	
Serum albumin (gm/dl)	No. of cases	CH_{50} (U/ml) Mean $\pm$ S D	AP_{50} (U/ml) Mean $\pm$ S D	C3 (mg/dl) Mean $\pm$ S D
$<$ 2	2	5.24 $\pm$ 1.02	53.34 $\pm$ 1.73	38.00 $\pm$ 2.83
2 - 3	6	5.02 $\pm$ 2.44	53.86 $\pm$ 11.20	54.67 $\pm$ 19.09
3 - 4	34	6.23 $\pm$ 3.72	66.65 $\pm$ 24.96	73.04 $\pm$ 33.39
4 - 5	12	6.86 $\pm$ 2.85	89.88 $\pm$ 12.58	107.67 $\pm$ 37.21

$r_{1,2}$ = 0.299 (p $<$ 0.05)

$r_{1,3}$ = 0.107 (p $>$ 0.05)

$r_{1,4}$ = 0.828 (p $<$ 0.001)

$r_{2,3}$ = -0.222 (p $>$ 0.05)

$r_{2,4}$ = 0.345 (p $<$ 0.05)

$r_{3,4}$ = -0.049 (p $>$ 0.05)

Table depicts that definite trend of rise was seen
in CH_{50} and C3 values in relation to serum albumin values.
Correlation coefficients (r) of serum albumin with CH_{50} and
C3 were observed to be 0.299 and 0.828 respectively, being

significant ($P \angle 0.05$ and $\angle 0.001$ respectively). However,
a non-significant ($P \ulcorner 70.05$) correlation ($r = 0.107$) was
found between serum albumin and AP_{50} values.

On further evaluation, it was observed that there
was a positive correlation ($r = 0.348$) of C3 with CH_{50}
values, being statistically significant ($P \angle 0.05$). On
comparison of C3 values with AP_{50} values, correlation
coefficient (r) was found to be -0.039, being non-significant
($P \ulcorner 70.05$). Similarly correlation between CH_{50} and AP_{50}
($r = -0.222$) was found to be non-significant ($P \ulcorner 70.05$).

Correlation of Haemoglobin Values with Complement Activity :

Mean values of CH_{50}, AP_{50} and complement C3 in
different groups of haemoglobin values are shown in Table XVII.

Table XVII

Correlation of haemoglobin values with complement activity.

Haemoglobin (gm/dl)	No.of cases	CH_{50}(U/ml) Mean ± S D	AP_{50}(U/ml) Mean ± S.D	C3 (mg/dl) mean ± S D
5 - 8	16	6.04 ± 3.44	54.69 ± 11.96	63.01 ± 22.04
8 - 11	17	5.00 ± 3.48	68.04 ± 38.48	64.76 ± 39.98
11 - 14	11	7.33 ± 3.00	63.76 ± 10.04	126.91 ± 34.04

$$r_{1,2} = 0.114 \quad (P \ulcorner 70.05)$$
$$r_{1,3} = 0.196 \quad (P \ulcorner 70.05)$$
$$r_{1,4} = 0.633 \quad (P \angle 0.001)$$

It is clear from Table that there was no definite
rising or declining trend in CH_{50} and AP_{50} values in relation

to haemoglobin values. Correlation coefficients(r) of haemoglobin values with CH_{go} and AP_{go} values were noticed to be 0.114 and 0.196 respectively, being non-significant ($P > 0.05$). On comparison of haemoglobin values with CS values, a positive correlation coefficient ($r = 0.638$) was observed, being statistically significant ($P < 0.001$).

...

DISCUSSION

The present work has been carried to study the complement activity in 32 pre-school children (1-5 years age) suffering from protein-calorie malnutrition and 12 nutritionally normal age matched children, serving as control. The study was conducted at M.L.B. Medical College, Jhansi between May 1981 and March 1982.

The primary aim of our study was to evaluate the complement profile in children suffering from PCM and compare the values with those obtained in control cases. Besides evaluating the complement activity traditional parameters viz. weight, length/height and mid-arm circumference, serum albumin and blood haemoglobin were used to assess the nutritional status of children. Complement activity was assessed by total haemolytic complement (CH_{50}) activity, alternative pathway activity (AP_{50}) and C3 concentration. It was also our endeavour to ascertain the possible inter-relationship between the clinical progress, following nutritional rehabilitation and the subsequent change in complement activity. With the objective in view, complement activity, anthropometric measurements, serum albumin and blood haemoglobin values were evaluated at the time of initial contact and in subsequent follow ups at 3 weeks, 4-7 weeks and 10-12 weeks interval. Statistical analysis was done to derive means and standard deviations (SD). An attempt was made to correlate age, weight, length/height,

mid-arm circumference, serum albumin and blood haemoglobin with various parameters of the complement activity.

The PCM group in our study was further classified into 19 cases of marasmus, 12 cases of marasmic-kwashiorkor and 3 cases of kwashiorkor according to Welcome classification. All the cases belonged to low socioeconomic status and most of them were suffering from gastrointestinal and respiratory tract infections. Care was however taken to exclude the cases in whom secondary factors thought to affect complement activity could have been operational. History of past illness and family history were noted in each case. All the children at the initial contact were receiving diet, grossly deficient in calories and proteins. None of the twelve control cases was suffering from any demonstrable illness at the time of inclusion in this study.

Based on observations depicted in tables I to XVII, various inferences have been drawn and discussed under different headings.

ANTHROPOMETRIC PROFILE :

Anthropometric profile of both the control and PCM cases at the time of initial contact (Table III) revealed that the mean weight, length (height) and mid-arm circumference in all 3 groups of PCM viz. marasmus, marasmic-kwashiorkor and kwashiorkor were appreciably less than those in healthy controls. However, groups of PCM among themselves did not show clear differences in anthropometric values. Subsequently,

during follow up, after nutritional rehabilitation and
subsidence of infection, it was seen that mean weight and
mid-arm circumference increased consistently during 1st, 2nd
and 3rd follow ups in cases of marasmus and marasmic-
kwashiorkor and during the 1st follow up in a single case of
kwashiorkor.

It was also observed that the sustained improvement
in weight and mid-arm circumference was more marked in cases
of marasmus. However, there was no improvement seen in mean
length (height) at subsequent follow ups in the 3 groups of
PCM cases, exception being a single case of kwashiorkor.
These findings are not surprising since it is known that
body weight is a more sensitive indicator of nutrition and
that evaluation of linear body change is usually possible at
longer intervals of time.

**Correlation of Weight (expressed as % of 50th percentile of
Harvard Standard) with Complement Activity :**

From Table XIII it is obvious that no definite trend
of rise or decline was seen in CH$_{50}$ values in relation to change
in percentage weight. Correlation coefficients of percentage
weight with CH$_{50}$ and AP$_{50}$ were 0.192 and 0.099 respectively,
being non-significant. However, a positive significant
correlation (r = 0.608) was seen between the percentage
weight and C3 values.

**Correlation of length/Height (expressed as % of 50th percen-
tile of Harvard Standard) with Complement Activity :**

Correlation coefficient of length (height) percentage

with both CH_{50} and AP_{50} values was non-significant, while C3 values (r = 0.636) was statistically significant, as shown in Table XIV.

Correlation of Mid-Arm Circumference with Complement Activity:

The correlation of the mid-arm circumference with complement activity (Table XV) revealed that there was no definite rise or decline in CH_{50} and AP_{50} values with the change in mid-arm circumference, correlation coefficients(r) being 0.265 and 0.142 respectively. As with weight and length/height, it was observed that there was a definite rising trend in C3 values in relation to arm circumference, correlation coefficient being 0.721.

Thus we see that the percentage of weight, height (length) and mid-arm circumference had a positive and significant correlation with complement C3 levels, while CH_{50} and AP_{50} values had no significant correlation with anthropometric measurements. Our observations are comparable with the findings of other workers in the field. Olusi et al (1976) in their study to evaluate the complement profile in PCM cases, noted that with refeeding, C3 was the first complement component to show a significant rise, followed by C9 and C6, thereby concluding that out of all the complement components, C3 was the most sensitive index of nutritional status. Kielmann et al (1976), in their study of complement profile in pre-school children has also shown a positive correlation of weight, length (height) and arm circumference with C3 values. Thus the findings of the present study and that of other workers clearly reveal that weight and length (height)

percentage, and arm circumference show a positive correlation with only one parameter of complement activity viz. C3 levels, suggesting that with nutritional rehabilitation, there is not only an improvement in the growth and development, but also an improvement in the immunological status of the child.

SERUM ALBUMIN AND HAEMOGLOBIN VALUES :

It is obvious from Table IV that the mean serum albumin and haemoglobin values were significantly depressed in PCM cases as compared to the controls (P $<$ 0.001). Similar findings have been observed by other workers in the field (Olusi et al, 1976 and Haller et al, 1978). Further it was observed that mean serum albumin and haemoglobin values were lowest in cases suffering from kwashiorkor (Table V and Fig. 8) at the initial contact. After nutritional repair there was a sustained rise in mean serum albumin levels, during the 3 follow ups, marasmus, marasmic-kwashiorkor cases. Similarly rise in serum albumin was seen during the 1st follow up in a single case of kwashiorkor (Table IX, X and XI). Like serum albumin level, mean haemoglobin level also increased substantially in the all 3 follow ups in marasmus and marasmic-kwashiorkor and during the 1st follow up in single case of kwashiorkor. Thus it was apparent that nutritional repair, with adequate amount of calories and proteins, led to a sustained rise of serum albumin and haemoglobin values in all groups of PCM. Evaluation of these two parameters of nutrition and their

correlation with the complement activity has not hitherto been attempted by other workers and hence comparison to other studies could not be done.

COMPLEMENT PROFILE IN STUDY GROUPS :

I CONTROLS :

Total Haemolytic Complement (CH_{50}) Activity :

In the present study, total haemolytic complement (CH_{50}) had mean values of 7.16 ± 1.92 U/ml. It was seen that values from our study were much lower than those obtained by other workers. Smythe et al (1971) found CH_{50} values in the range of 1/128 - 1/512. Chandra (1972) and Jagadeesan and Reddy (1979) reported mean values of 58.00 ± 12.00 and 66.10 ± 8.21 U/ml respectively. However, Suskind et al (1976) found very high values of CH_{50} viz. 350.00 ± 50.00 U/ml in their study. The possible explanation for wide variation in CH_{50} values, obtained by various authors, could be that estimation of CH_{50} depends upon the standardization techniques used in different laboratories.

Since all control cases in our study were free of infections, we could not predict the effect of infection on the value of CH_{50} in well-nourished children.

Alternative Pathway Activity (AP_{50}) :

In our study mean value of AP_{50} were found to be 64.70 ± 10.11 U/ml. Inspite of our best efforts reference values of AP_{50} (a measure of alternative pathway activity) could not be found in literature for comparative study.

Complement C3 Values :

C3 complement values (mg/dl), considered to be most sensitive index of complement activity, had mean values of 125.63 ± 23.96 in normal control children. These values were found to be consistent with those obtained by various other workers viz. Chandra (1972), Sirisinha et al (1973), Chandra (1975), Olusi et al (1976), Kielmann et al (1976), Haller et al (1978) and Jagadeesan and Reddy (1979). However Neumann et al (1975) found slightly lower values of C3 i.e. 86.90 ± 2.40 mg/dl.

Chandra (1975), in his study to evaluate the serum complement levels in malnutrition and well-nourished children, observed that the complement C3 value was much higher (245.00 ± 57.00 mg/dl) in well-nourished children with infection than the other group of well-nourished children without infection (133.00 ± 18.00 mg/dl). The author however could not ascribe any explanation of the hightened C3 levels with infection in well-nourished children. Since all the 12 control children in our study were free of infection, we could not arrive at a definite conclusion, regarding the effects of infections on C3 complement levels.

II PROTEIN-CALORIE MALNUTRITION :

INITIAL CONTACT :

Total Haemolytic Complement (CH_{50}) Activity :

It is evident from Table IV that at the time of initial contact, mean CH_{50} value in the PCM group was lower

than the value in control group, the difference between the two values was statistically non-significant ($p > 0.05$).

On further analysis it was observed that though the values of CH_{50} in marasmus, marasmic-kwashiorkor and kwashiorkor were lower than the controls (Table V and Fig. 6), these values were not significantly different from the controls ($p > 0.05$). Similarly the group differences of mean CH_{50} values were not appreciably different from each other. In contrast to our study, Smythe et al (1971) and Chandra (1975) reported significantly lowered values of CH_{50} in infants and children with PCM. Suskind et al (1976), in an elaborate study to assess the complement activity in 28 children with severe PCM, using the CH_{50} titre as the parameter, reported a significant depression of CH_{50} levels, only in cases of kwashiorkor, as compared to the controls. However, on comparing the CH_{50} values of children with marasmus and marasmic-kwashiorkor to those of controls, they did not observe any significant difference. Haller et al (1978) and Jagadeesan and Reddy (1979) have also observed a more marked depression of CH_{50} values in kwashiorkor than in marasmus and marasmic-kwashiorkor cases.

The results of the present study therefore reveal, that, though CH_{50} values were lower in all groups of PCM, a statistically significant difference as compared to controls, could not be elucidated. Further, unlike other workers, we could not arrive at any correlation between the CH_{50} values and the severity of PCM; values in all 3 groups remained

more or less equal. This was in contrast to the results
obtained by other workers, where not only CH_{50} values in PCM
were statistically different from the controls but maximum
depression was also observed in the most severe form of PCM
viz. kwashiorkor.

Alternative Pathway Activity (AP_{50}) :

It is evident from Table IV that the mean AP_{50}
values in the PCM group was not statistically different from
the controls. Further the mean AP_{50} values in marasmus, mara-
smic-kwashiorkor and kwashiorkor were found to 63.02 ± 24.91,
62.32 ± 22.33 and 51.40 ± 3.90 respectively (Fig. :7). Although
these values in 3 groups of PCM were lower, only the value
in kwashiorkor group was significantly lower ($p < 0.05$) as
compared to the controls. This suggests that the alternate
pathway activity was adversely affected only in the
kwashiorkor group, values being unaffected in marasmus and
marasmic-kwashiorkor cases. Since there is paucity of data
regarding AP_{50} in literature, a comparison of these values
could not be ascertained.

However, Sirisinha et al (1973) and Heller et al
(1973) studied the alternative pathway activity by evaluating
the concentration of factor B (named C3 pro-activator, pre-
viously) and observed that this factor was depressed in
children suffering from PCM. Since these authors found
normal C4 levels, which are depressed in classical pathway
activity, they suggested that in PCM, alternative pathway
was also activated. However, in the present study, alterna-
tive pathway activity was found to be unaffected except in
kwashiorkor. The possible mechanism of depressed AP_{50}
(alternative pathway activity) values could be that

infection triggered alternative pathway thereby resulting
in diminished concentration of AP_{50} values. Since in our
study, all the cases of kwashiorkor were severely infected,
maximum depression of AP_{50} values was obtained in this group.
Further, a significant reduction in alternative pathway
activity could have accounted for lowering the antimicrobial
resistance therefore resulting in severe infection in
kwashiorkor.

Complement C3 Values :

The complement C3 levels in PCM group had mean value
of 60.59 ± 20.25 mg/dl. These values were found to be statisti-
cally lower than those in controls (Table IV). On further
analysis of the C3 levels in marasmus, marasmic-kwashiorkor
and kwashiorkor it was observed that the values in all 3
groups were significantly lower as compared to controls
($p < 0.001$), maximum depression being observed in cases of
kwashiorkor. However, the mean values in the 3 groups of
PCM were not appreciably different from each other (Table V
and Fig. 8). A significant depression of C3 level in PCM
cases was also obtained by various other workers in the field
viz. Chandra (1972), Sirisinha et al (1973), Chandra (1975),
Neumann et al (1975), Olusi et al (1976), Kielmann et al
(1976), Haller et al (1978), Kielmann and Curris (1979) and
Jagadeesan and Reddy (1979).

It was further observed in the present study that
a definite correlation existed between the serum albumin
concentration and complement C3 levels ($r = 0.625$). In the

kwashiorkor group concentration of serum albumin was the
lowest and maximum depression of the C3 level was also
obtained in these cases (Table V), thus suggesting a direct
correlation of serum C3 values with the severity of mal-
nutrition.

Correlation of Age with Complement Activity :

No definite trend of rise or decline was seen in
complement values viz. CH_{50}, AP_{50} and C3 in relation to age,
thus suggesting no effect of age on complement activity.
This aspect has not been evaluated so far by any other
worker.

Correlation of Serum Albumin Values with Complement Activity :

It is evident from Table XVI, that when serum albumin
levels were correlated with the CH_{50}, AP_{50} and C3 levels, a
definite and positive rise was observed only in CH_{50} and C3
levels with the increasing levels of albumin. Correlation
coefficients (r) of serum albumin with CH_{50} and C3 were
0.399 and 0.625 being significant at P < 0.05 and < 0.001
respectively. However, no rise in AP_{50} values was observed
with the increasing levels of serum albumin (r = 0.107).

On further evaluation, it was observed that there
was a positive correlation (r = 0.546) of C3 with CH_{50}
values, being statistically significant (P < 0.05). However
on comparison of C3 and CH_{50} values with AP_{50} values,
correlation coefficients (r) were found to be -0.039 and
-0.322 respectively, both being non-significant (> 0.05).

A positive significant correlation between C3 and CH_{50} values implies that a change in the concentration of C3 will reflect on CH_{50} values. In this regard Spitzer(1977b) reported that CH_{50} values were affected only when there was approximately 80% decrease in C3 levels. However, since negative non-significant correlation was observed between C3 and AP_{50} values, the values of these two parameters would thus be independent of each other in PCM cases.

A similar significant correlation between serum albumin levels and complement activity was also observed by Sirisinha et al (1973), Haller et al (1978) and Jagadeesan and Reddy (1979). They reported that there was a direct correlation between the degree of complement depletion (especially C3 levels) to the severity of the depletion of various plasma proteins.

Sirisinha et al (1973), Haller et al (1978) and Jagadeesan and Reddy (1979) thus suggested that decreased protein synthesis in the liver played a major role in the reduction of C3 levels and hence in the impairment of complement system in PCM. However, Kielmann and Curcio (1979) showed that there was no significant correlation between total serum proteins and C3 levels.

Correlation of Haemoglobin Values with Complement Activity :

An attempt was made to correlate the haemoglobin values with the complement activity (Table XVII). We observed that there was no definite rising or declining trend

in CH_{50} and AP_{50} values in relation to haemoglobin values, correlation coefficients (r) being 0.114 and 0.196 respectively (P > 0.05). However, a significant positive correlation (r = 0.628) was observed between haemoglobin and C3 values.

A significant correlation between haemoglobin and C3 values in our study suggest that haemoglobin per se, could also be one of the factors responsible for depression of C3 levels in PCM cases. However, a study conducted by Kielmann and Curcio (1979) did not show any significant correlation of C3 levels with haemoglobin values.

Complement Activity and Infection :

In the present study since all the PCM cases were suffering from infection, it was not possible to elucidate whether there was any subsequent difference in the complement activity between infected and non-infected cases. Sirisinha et al (1973), Chandra (1975), Smskind et al (1976) and Kielmann and Curcio (1979) have all reported that there was pronounced depression of complement activity (especially CH_{50} and C3 levels) in PCM children suffering from infection than in those without it. Further they also reported difference in the complement levels with severity of infection, levels being lower in severely infected cases than in those having mild infection.

FOLLOW UP :

After the initial assessment of anthropometry,
serum albumin, haemoglobin and complement activity in the
PCM cases, all of them were put on nutritional rehabilita-
tion schedule and infections were treated by appropriate
antibiotics. An attempt was made to follow all PCM cases
with repeat anthropometry, estimation of serum albumin,
haemoglobin and complement activity.

Total Haemolytic Complement (CH_{50}) Activity :

It is evident from Tables IX, X and XI that after
nutritional repair, CH_{50} levels attained maximum mean values
after the 1st follow up in all the cases of marasmus and
marasmic-kwashiorkor cases and also in a single case of
kwashiorkor. Although in the 2nd and 3rd follow up, mean
levels had a declining trend in both marasmus and marasmic-
kwashiorkor cases, yet the levels remained higher than that
obtained at initial contact. Since the solitary case of
kwashiorkor left the hospital after 1st follow up, subsequent
CH_{50} level could not be ascertained. Our results are consis-
tent with the findings of Chandra (1975), Sushind et al(1976)
and Jagadeesan and Reddy (1979).

Alternative Pathway Activity (AP_{50}) :

Tables IX, X and XI clearly demonstrate that on
evaluation of AP_{50} values at follow up in different groups
of PCM, no consistent pattern of rise was observed. Since
there is a paucity of available literature on AP_{50} values,
comparison of our values could not be elucidated.

Complement C3 Values :

It is evident from Table IX, X and XI, and Fig. 9, 10 and 11 that after nutritional rehabilitation C3 values, attained maximum mean level after the 1st follow up, in only marasmic-kwashiorkor. Values in marasmus showed a definite increasing trend till the 2nd follow up. In a single case of kwashiorkor followed only once, significant rise of C3 level was observed. Rise of C3 levels after nutritional rehabilitation was also observed by Sirisinha et al (1975), Chandra (1975), Olusi et al (1976) and Jagadeesan and Reddy (1979).

A critical analysis of the complement activity on follow up in the 3 groups of PCM revealed that the levels of most complement components, after nutritional rehabilitation, rose to above control values. The mechanism involved in such overshoot or rebound is still not known. However, Sirisinha et al (1975) are of the view that after complement depletion in PCM, there is an accentuated synthesis of the complement proteins and a state of over production ensues, accounting for above the normal values of complement components.

Thus the findings on the complement activity in children suffering from protein-calorie malnutrition, in the present study, reveal certain interesting observations. That the complement profile was significantly depressed in PCM cases as compared to the controls. It was seen that the

depression of complement activity was mainly accounted
by a significant depression of the complement C3 level,
while CH_{50} and AP_{50} values, though lower were not statisti-
cally significant from the controls. Another significant
finding of our study was highly significant depression of
complement C3 ($P < 0.001$) in all 3 groups of PCM, levels
being maximally depressed in kwashiorkor. However, though
CH_{50} values were lower in all the groups, no statistical
significance was observed as compared to the controls
($P > 0.05$). AP_{50} values, on the other hand, were found to
be depressed only in kwashiorkor ($P < 0.05$), while no
significant difference was obtained in marasmus and marasmic-
kwashiorkor. Since C3 levels were found to be depressed in
all forms of malnutrition, it was inferred that probably
complement C3 was the most sensitive index of nutritional
status. The findings, that CH_{50} levels were more or less
equal in all the groups of PCM and also did not show any
significant difference from the controls, demonstrated that
this parameter was not a very good index of evaluation of
complement activity, a change in CH_{50} levels could possibly
be affected by at least a 50% reduction in C3 levels
(Spitzer, 1977b).

In the present series an attempt was made to evaluate
the various factors which could possibly have an adverse
influence on the complement profile. On the basis of a
positive correlation of nutritional status (measured by

anthropometric indices), serum albumin and haemoglobin
values with the complement activity, we arrived at the
following possible explanations for the depression of
complement system :

1. <u>Reduction in Protein Synthesis</u> :

This was the single most important factor as
observed in present study to cause depression of complement
activity. This finding is substantiated by the following
observations :

(a)   There was a uniform depression of serum albumin
levels in all the groups of PCM. Further it was observed
that greater the reduction of albumin levels, more signifi-
cant was the depression of complement activity, the
correlation coefficient between serum albumin and C3 levels
being highly significant (P $\angle$ 0.001).

(b)   With nutritional repair, there was not only a
substantial increase in serum albumin levels but also
concomitant rise in complement activity.

These observations in our study suggested that
nutritional status of the individual could influence the
complement system.

2. <u>Reduction in Haemoglobin Values</u> :

Since a definite correlation was observed between
haemoglobin levels and complement C3 activity, one could
suggest that haemoglobin levels played an important role in
the depression of complement levels.

Various hypothesis have been put forward by different workers to explain the possible mechanisms of depression of complement activity in PCM. Sirisinha et al (1973), Chandra (1975) and Suskind et al (1976) are unanimous in their opinion that the child's nutritional status was probably the major factor in causing the depression of complement activity. Chandra (1975) further stated that since liver damage occurred in PCM and liver was the main site of C3 synthesis, reduction of C3 levels could be a corrollary to liver damage. Another important factor to cause depression of complement activity could be a phenomenon of complement consumption which occurred in the presence of infection. Suskind et al (1976) suggested that anticomplementary activity in serum of PCM cases could also contribute to depression of CH_{50}. Other possible explanations for depression of complement system in malnourished children, though unimportant, could be the changes in blood vascular compartment occuring in malnutrition (Chandra, 1975). Complement depletion could also occur as a result of protein-losing gastroenteropathy in PCM cases (Chandra, 1975).

...

++
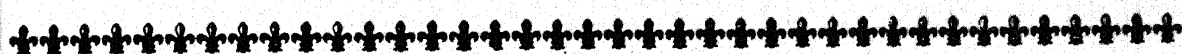
SUMMARY & CONCLUSION

++
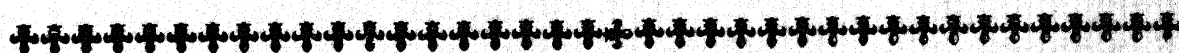
The present study on complement system in children with protein-calorie malnutrition was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi (U.P.). Thirty two pre-school children (1-5 years age) suffering from protein-calorie malnutrition and twelve age matched well-nourished healthy controls comprised the material for the present study. Complement activity was assessed by three parameters viz. total haemolytic complement (CH_{50}) activity, alternative pathway activity (AP_{50}) and serum complement C3 levels. Besides complement activity, various anthropometric measurements (weight, length/height and mid-arm circumference), serum albumin and blood haemoglobin values were noted in each case.

Children suffering from PCM were treated and put on nutritional rehabilitation schedule. An attempt was made to follow the cases at 3 weeks, 4-7 weeks and 10-12 weeks interval.

For the purpose of analysis children suffering from PCM were divided into 3 groups viz. marasmus, marasmic-kwashiorkor and kwashiorkor according to McLaren classification.

From the data collected means, standard deviations and correlation coefficients were calculated. Means of PCM cases were compared and discussed in relation to controls.

ANTHROPOMETRIC PROFILE :

Anthropometric values viz. weight, length/height and mid-arm circumference in children suffering from PCM were appreciably less than in controls. However, different groups of PCM as per McLaren classification, did not reveal any clear differences.

Following nutritional rehabilitation, mean weight and mid-arm circumference increased consistently during 1st, 2nd and 3rd follow ups in cases of marasmus and marasmic-kwashiorkor and during the 1st follow up in a single case of kwashiorkor. However, there was no improvement seen in mean length (height) at subsequent follow ups in all the 3 groups of PCM, exception being a single case of kwashiorkor.

SERUM ALBUMIN VALUES :

Mean serum albumin level (3.13 ± 0.80 gm/dl) was found to be significantly depressed in PCM as compared to the controls (4.06 ± 0.37 gm/dl).

Mean serum albumin levels (gm/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor were 3.49 ± 0.83, 2.72 ± 0.41 and 2.00 ± 1.62. These values were significantly lower than those of controls. Value was lowest in cases suffering from kwashiorkor.

Following nutritional rehabilitation there was increase in mean serum albumin levels in all 3 PCM groups.

HAEMOGLOBIN VALUES :

Mean haemoglobin level was significantly lower in PCM cases (7.82 ± 1.62 gm/dl) as compared to controls (12.07 ± 1.06 gm/dl).

Mean haemoglobin values (gm/dl) i.e. 8.62 ± 1.14, 6.96 ± 1.60 and 5.60 ± 0.53 in marasmus, marasmic-kwashiorkor and kwashiorkor, being lowest in last group, were also significantly lower as compared to controls.

During follow up, as with serum albumin levels, there was a definite rise in mean haemoglobin value in each of the 3 groups of PCM.

COMPLEMENT ACTIVITY :

Total Haemolytic Complement (CH_{50}) Activity :

Mean CH_{50} value (U/ml) in PCM cases (5.89 ± 3.45) was not significantly different from controls (7.16 ± 1.92).

Mean CH_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor were found to be 5.51 ± 3.66, 5.77 ± 2.70 and 5.48 ± 3.96 respectively. These values were not significantly different from the controls. Also, the group differences of mean CH_{50} values were not appreciably different.

During follow up there was no constant pattern of rise or decline in mean CH_{50} values in marasmus and marasmic-kwashiorkor cases. However, in single case of kwashiorkor followed only once, there was an increase in CH_{50} value by 3.58 U/ml.

Alternative Pathway Activity (AP_{50}) :

Mean AP_{50} value (U/ml) in PCM cases (61.68 ± 23.07) was not significantly different from controls (64.70 ± 10.11).

Mean AP_{50} values (U/ml) in 3 group of PCM viz. marasmus, marasmic-kwashiorkor and kwashiorkor were found to be 63.82 ± 24.91, 62.32 ± 22.53 and 51.40 ± 3.90 respectively. Out of all these values, mean value in kwashiorkor group was significantly lower as compared to controls. However, there were no significant differences of AP_{50} means within the PCM groups.

During follow up periods, there was no constant difference in repeat AP_{50} values in marasmus and marasmic-kwashiorkor. However, in a single case of kwashiorkor value decreased from 47.12 U/ml to 45.00 U/ml after 1st follow up.

Complement C3 Values :

Mean complement C3 value in children suffering from PCM (60.89 ± 23.25 mg/dl) was significantly lower as compared to controls (125.63 ± 23.98 mg/dl).

Mean C3 values (mg/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were found to be 63.16 ± 23.96, 62.30 ± 23.48 and 39.67 ± 2.31 respectively. These values were significantly lower as compared to controls. However, mean values in 3 groups were not significantly different from each other.

In children suffering from marasmus mean C3 values rose consistently even above control levels, after 1st and 2nd follow up. In a single case, followed 3rd time, a rise from 83.00 mg/dl to 114 mg/dl was noticed.

During follow up in marasmic-kwashiorkor cases, though C3 values attained a significantly higher levels, there was no consistent rising pattern.

In kwashiorkor group, a rise of 52.00 mg/dl in C3 value was observed in 2 weeks time in a single case followed.

CORRELATION OF DIFFERENT PARAMETERS WITH COMPLEMENT ACTIVITY:

Age with Complement Activity :

No definite trend of rise or decline was seen in complement values viz. CH_{50}, AP_{50} and C3 in relation to age.

Weight (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

No definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to weight percentage. However, a significant positive correlation ($r = 0.685$) was observed between weight percentage and C3 values.

Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

There was non-significant correlation seen between percentage length (height) and CH_{50} as well as AP_{50} values. However, a positive significant correlation ($r = 0.636$) was seen between length percentage and C3 values.

Mid-Arm Circumference with Complement Activity :

No definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to mid-arm circumference. However, there was positive significant correlation ($r = 0.721$) between mid-arm circumference and C3 values.

<u>Serum Albumin Values with Complement Activity</u> :

   Correlation coefficients (r) of serum albumin
with CH_{50} and C3 values were 0.299 and 0.825 respectively,
being significant. However, there was no significant
correlation between serum albumin and AP_{50} values.

<u>Haemoglobin Values with Complement Activity</u> :

   There was no definite rising or declining trend
in CH_{50} and AP_{50} values in relation to haemoglobin values.
However, there was a significant positive correlation
(r = 0.628) between haemoglobin and C3 values.

<u>Correlation among CH_{50}, AP_{50} and Complement C3 Values</u> :

   A positive significant correlation (r = 0.348)
was observed between CH_{50} and C3 values. However, no
significant correlation was found between CH_{50} and AP_{50}
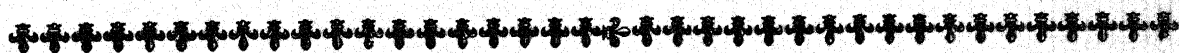as well as between C3 and AP_{50} values.

<u>COMPLEMENT ACTIVITY AND INFECTION</u> :

   Since all control cases were free from infection
and every PCM case had one or the other type of infection,
we could not predict the effect of infection on the
complement activity.

     ***

BIBLIOGRAPHY

1. Allison AC. Interactions of antibodies, complement components and various cell types in immunity against viruses and pyogenic bacteria. Transplant Rev 1974; 19 : 3.

2. Arnhold R. The arm circumference as a public health index of protein-calorie malnutrition of early childhood. The Quac stick : a field measure used by the Quaker Service team in Nigeria. J Trop Pediatr 1969; 15 : 243.

3. Austen KF, Becker EL, Borsos T et al. Nomenclature of complement. Bull WHO 1968; 39 : 935.

4. Bengoa JM. The problem of malnutrition. WHO Chron 1974; 28 : 3.

5. Bistrian BR, Blackburn GL, Hallowell E. Protein status of general surgical patients. J A M A 1974; 230 : 858.

6. Bordet J. Ann Inst Pasteur (Paris) 1896; 12 : 688.

7. Chandra RK. Immunocompetence in undernutrition. J Pediatr 1972; 81 : 1194.

8. Chandra RK. Serum complement and immunoconglutinin in malnutrition. Arch Dis Child 1975; 50 : 225.

9. Dias da Silva W, Eisele JW, Lepow IH. Complement as a mediator of inflammation, III. Purification of the activity with anaphylatoxin properties generated by interaction of the first four components of complement and its identification as a cleavage product of C3. J Exp Med 1967; 126 : 1027.

10. Edelman R, Suskind R, Sirisinha S, Olson R.
    Mechanisms of defective delayed cutaneous hypersensi-
    tivity in children with protein-calorie malnutrition.
    Lancet 1973; 1 : 506.

11. Ehrlich P, Morgenroth J. Berl Klin Wschr 1899; 36:481.

12. Fahey JL, McKelvey EM. Quantitative determination of
    serum immunoglobulins in antibody-agar plates. J
    Immunol 1965; 94 : 84.

13. FAO/WHO Expert Committee on Nutrition. Eight Report.
    WHO Tech Rep Ser, Geneva 1971, No 477.

14. Funt O. The biological role of the complement system
    and the clinical importance of complement measurements.
    Haematologia 1978/1979; 12 (1-4) : 85.

15. Gewurz H. Alternative pathways to activation of the
    complement system. In Biological Activities of
    Complement. Ed Ingram DG. Basel, Karger, 1972.

16. Ghai OP. Mortality of severe cases of protein-calorie
    malnutrition in hospitals. Indian Pediatr 1975; 12 : 79.

17. Ghai OP. Essentials of Pediatrics 1st ed. New Delhi.
    Sagar Publications, 1977, p 89.

18. Ghai OP, Chaudhri SN, Jaiswal VN, Sinclair S.
    Nutritional assessment of pre-school children of a
    rural community. Indian J Med Res 1970; 58 : 1631.

19. Ghosh S. The Feeding and Care of Infants and Young
    Children 4th ed. New Delhi. Voluntary Health Associa-
    tion of India, 1981, p 1.

20. Gigli I, Nelson RA Jr. Complement dependent immunophagocytosis.i. Requirements for C'1, C'4, C'2, C'3. Exp Cell Res 1968; 51 : 45.

21. Gomez F, Ramos-Galvan R, Cravioto JM, Frenk S. Malnutrition in infancy and childhood with special reference to kwashiorkor. Adv Pediatr 1955; 7 : 131.

22. Gopalan C. The nutrition problem in India. J Indian Med Assoc 1974; 62 : 234.

23. Gotze O, Muller-Eberhard HJ. The C3 activator system : an alternative pathway of complement activation. J Exp Med 1971; 134 : 905.

24. Grabar P, Williams CA. Methode permettant l'etude conjuguee des proprietes electrophoretiques et immunochimiques d'un melange de proteines. Application au serum sanguin. Biochim biophys Acta 1953; 10 : 193.

25. Haller L, Zubler RH, Lambert PH. Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. Clin Exp Immunol 1978; 34 : 248.

26. Jagdeesan V, Reddy V. Serum complement levels in malnourished children. Indian J Med Res 1979; 70:745.

27. Jelliffe DB. Protein-calorie malnutrition in tropical pre-school children : a review of recent knowledge. J Pediatr 1959; 54 : 227.

28. Jelliffe DB. Assessment of the Nutritional Status of the Community. WHO Monogr Ser, 1966, No. 53.

29. Jelliffe DB, Dunn G, Stuart KL. Kwashiorkor and marasmus in Jamaican infants. W Indian Med J. 1954; 2 : 43.

30. Johnston RB Jr. Immunologic system. In Textbook of Pediatrics. Ed Nelson WE, Vaughan VC, Mckay RJ, Behrman RE. Philadelphia London Toronto, W B Saunder's Company. Tokyo, Igaku Shoin Ltd., 1979, p 665.

31. Johnston RB Jr, Stroud RM. Complement and host defence against infection. J Pediatr 1977; 90 : 169.

32. Kanawati AA, Mclaren DS. Assessment of marginal malnutrition. Nature 1970; 228:573.

33. Keilmann AA, Curato IM. Complement (C3), nutrition and infection. Bull WHO 1979; 57 (4) : 113.

34. Keilmann AA, Uberoi IS, Chandra RK, Mehra VL. The effect of nutritional status on immune capacity and immune response in pre-school children in a rural community in India. Bull WHO 1976; 54 : 477.

35. Koopman WJ, Sandberg AL, Wahl SM et al. Interaction of soluble C3 fragments with guinea pig lymphocytes; comparison of effects of C3a, C3b, C3c and C3d on lymphokine production and lymphocyte proliferation. J Immunol 1976; 117 : 331.

36. Kumar KK, Agrawal T, Yadav SK, Dhanija JP. A study of cell mediated immune response in protein-calorie malnutrition. Indian Pediatr 1978; 15 : 303.

37. Lachmann PJ, Coombs RRA. Complement conglutinin and immunoconglutinin. In Complement : A Ciba

Foundation Symposium. Ed Wolstenholme GEW, Knight
J London, J & A Churchill Ltd., 1965; p 281.

38. Laurell CB. Quantitative estimation of proteins by
electrophoresis in agarose gel containing antibodies.
Analyt Biochem 1966; 15 : 45.

39. Mancini G, Carbonara AO, Heremans JF. Immunochemical
quantitation of antigens by single radial immuno-
diffusion. Immunochemistry 1965; 2 : 235.

40. Mayer MM. Complement and complement fixation. In
Experimental Immunochemistry. Ed Kabat Thomas EA.
Illinois, Springfield, 1961, p 133.

41. McConnell I, Lachmann PJ. Complement and cell
membranes. Transplant Rev 1976; 32 : 72.

42. McLaren DS, Burman D. Textbook of Paediatric Nutrition
1st ed, Edinburgh. Churchill Livingstone, 1976;108 .

43. McLaren DS, Pellett PL, Read WWC. A simple scoring
system for classifying the severe forms of protein-
calorie malnutrition in early childhood. Lancet
1967; 1 : 533.

44. Miller DJ, Oldstone MBA, Cooper NR. Complement
dependent lysis of vesicular stomatitis virus.
Fed Proc 1976; 35 : 494.

45. Miller GW, Nussenzweig V. A new complement function:
solubilization of antigen-antibody aggregates. Proc
Nat Acad Sci (Wash.) 1975; 72 : 418.

(vi)

46. Newman CG, Lawlor GJ, Newton C, Herbert J, Ammann AJ, Jacob M. Immunologic response in malnourished children. Am J Clin Nutr 1975; 28 : 89.

47. Nutrition Sub-Committee of the Indian Academy of Pediatrics. Report of Convenor. Indian Pediatr 1972; 9 : 360.

48. Olusi SO, Mcfarlane H, Ade-Serrano M, Osunkoya BO, Adeniun W. Complement components in children with protein-calorie malnutrition. Trop Geogr Med 1976; 28 : 323.

49. Ouchterlony O. In vitro method for testing the toxin-producing capacity of diphtheria bacteria. Acta path microbiol Scand 1948; 25 : 186.

50. Pepys MB. Role of complement in the induction of immunological responses. Transplant Rev 1976; 32 : 93.

51. Pfeiffer R. Z Hyg Infektr 1894; 19 : 75.

52. Phillips I, Wharton B. Acute bacterial infections in kwashiorkor and marasmus. Br Med J 1968; 1 : 407.

53. Platts-Mills TAE, Ishizaka K. Activation of the alternative pathway of human complement by rabbit cells. J Immunol 1974; 113 : 348.

54. Puri V, Misra PK, Saxena KC, Saxena PK, Saxena SP, Agarwal GG. Immune status in malnutrition. Indian Pediatr 1980; 17 : 127.

55. Ramalingaswami P, Ramalingaswami V. India. In Health Service Prospects : An International Survey. ed Douglas - Wilson I, McLachlan G, London, Lancet and the Nuffield Provincial Hospitals Trust, 1973, p 183.

56. Rao KSJ. Protein-calorie malnutrition. Indian J
    Med Res 1978; 68 (Suppl) : 17.

57. Rao NP, Singh D, Swaminathan MC. Nutritional status
    of pre-school children of rural communities near
    Hyderabad city. Indian J Med Res 1969; 57 : 2132.

58. Reddy V, Bhaskaram C, Raghuramulu N, Immunological
    responses in malnourished children. Indian Pediatr
    1977; 14 : 255.

59. Reddy V, Srikantia SG. Interaction of nutrition and
    immune response. Indian J Med Res 1978; 68 (suppl) : 48.

60. Rother K. Leucocyte mobilizing factor. A new biological
    activity derived from the third component of complement.
    Eur J Immunol 1972; 2 : 550.

61. Ruddy S, Gigli I, Austen KF. The complement system
    of man (second of four parts). N Engl J Med 1972;
    287 : 545.

62. Scrimshaw NS, Taylor CF, Gordon JE. Interactions of
    Nutrition and Infection. WHO Monogr Ser, 1968; No.57.

63. Selvaraj RJ, Bhat KS. Metabolic and bactericidal
    activities of leucocytes in protein-calorie malnutri-
    tion. Am J Clin Nutr 1972; 25 : 166.

64. Seth V, Chandra RK. Opsonic activity, phagocytosis and
    intracellular bactericidal activity of polymorphs in
    undernutrition. Arch Dis Child 1972; 47 : 282.

65. Shin HS, Snyderman R, Friedmann E et al. Chemotaxis
    and anaphylatoxic fragment cleaved from the fifth
    component of guinea pig complement. Science 1968;
    162 : 361.

66.   Sirisinha S, Suskind R, Edelman R, Charupatana C,
       Olson RE. Complement and C3 proactivator levels in
       children with protein-calorie malnutrition and effect
       of dietary treatment. Lancet 1973; 1 : 1016.

67.   Smythe PM, Schonland M, Brereton-Stiles GG et al.
       Thymolymphatic deficiency and depression of cell
       mediated immunity in protein-calorie malnutrition.
       Lancet 1971; 2 : 939.

68.   Spitzer RE. The complement system. Pediatr Clin North
       Am 1977a; 24(2) : 341.

69.   Spitzer RE. Immunobiology and clinical importance of
       complement system of man. Adv Pediatr 1977 b; 24 : 43.

70.   Stevens MB, Maner RW, Asbrock T et al. Stimulation of
       neutrophil oxidative metabolism by the alternative
       pathway of complement activation : A mechanism for
       the spontaneous NBT test. Blood 1975; 45 : 843.

71.   Suskind R, Edelman R, Kulapongs P, Pariyanonda A,
       Sirisinha S. Complement activity in children with
       protein-calorie malnutrition. Am J Clin Nutr 1976;
       29 : 1089.

72.   Thompson RA. Techniques in Clinical Immunology.
       1st ed. Blackwell Scientific, 1977.

73.   Waterlow JC. Fatty liver disease in infants in the
       British West Indies. Spec Rep Med Res Coun (London)
       1948, No 263.

74.   Waterlow JC, Rutishauser IHE. Malnutrition in man.
       In Early Malnutrition and Mental Development. Ed.
       Cravioto J, Hambraeus L, Vahlquist B. Sweden, Almqvist
       and Wiksell, 1974, p 13.

75.   Williams CD. A nutritional disease of children
       associated with a maize diet. Arch Dis Child
       1933; 8 : 423.

...

SAMPLE CASE SHEET

CASE SHEET

NAME :   CASE NO.:

Date of birth :   Sex: Male/Female

Age :

Father's Name :

Address :

Occupation : Father-   Mother-

Total Income of Family: Rs.   /Month.

Per capita income   :   Rs.   /Month.

Birth order of child:

Geniological tree   :

Dietary History: Started   Upto   Dilution
                         at age   age

Breast Milk

Artificial Milk only

Added artificial milk

Solids added

Present diet :

Calories   Proteins   Adequate / Inadequate

IMMUNISATION HISTORY :

	Smallpox	B.C.G.	Polio(oral)	Triple(D.P.T.)
I				
II				
III				
IV				
V				

ANTENATAL & NATAL HISTORY :

Gest. period	Birth Wt.	Drug intake	Significant illness

NOTE :

POST NATAL HISTORY (First 4 weeks):

No problem   Fever Sepsis Jaundice   Cyanosis   Others.

MILE STONES (DEVELOPMENTAL BEHAVIOUR) :

MOTOR :   AGE :

1. Head control

2. Sitting

3. Crawling

4. Standing with support
         without support

5. Walking

6. Running

Comments if any.

MANIPULATIVE :

1. Grasp.

2. Self feeding : Spoon
                         Cup

3. Help in Dressing.

Comments if any.

SOCIAL :

1. Smile (Social)

2. Response to call by name

3. Sphincter control : Bladder   Day   Night
                              Bowel   Day   Night

Comments if any.

SPEECH :

1. Single word (Mamma etc.)

2. Jargon

3. Small broken sentences

4. Long sentences

Comments if any.

FAMILY HISTORY :

PRESENT ILLNESS :

PAST ILLNESS :

1. Category   i

2. Category   ii

3. Definite H/o Primary complex

4. H/o Pertusis

       Measles

       Worm infestation

Note :- Category i - H/o acute illness viz. fever, vomiting,
        diarrhoea, convulsion etc. lasting more than 4 days
        during the previous two weeks.
        Category ii- H/o cough, fever, vomiting, convulsion,
        diarrhoea etc. lasting more than 2 weeks any time
        during the previous six months.

CLINICAL EXAMINATION

GENERAL APPEARANCE :

    Healthy

    Malnourished

	DATE		

Psychomotor changes :

    Fretful

    Listless

HAIR :

    Normal

    Dyspigmentation

    Easy pluckability

    Sparseness

FACE :

    Moon Face

EYES :

    Conjunctival xerosis

    Bitot's spots

    Pale conjunctiva

MOUTH :

    Angular stomatitis

    Cheilosis

    Glossitis

    Swollen bleeding gums.

Dentition :

Thyroid gland :

    Goitre

SKIN :

    Oedema (Bilateral)

    Follicular hyperkeratosis

    (Type I)

    Pellagrous dermatosis

    Flaky paint dermatosis

    Diffuse depigmentation

    Mosaic dermatosis

Loss of Subcutaneous Fat :

    Muscle Wasting :

SKELETON :

    Epiphyseal enlargement
    (Wrist)

    Rickety Rosary

    Persistently open ant.
    font.

    Harrison's sulcus

    Bossing of skull

    Knock knees

    Bow legs.

DATE			

NOTE :

ABDOMEN :

    Liver

    Spleen

    Pot Belly

    Any other.

C.V.S. :

    Normal

    Any abnormality

RESPIRATORY SYSTEM :

    Normal

    Any abnormality

C.N.S. :

    Normal

    Any abnormality

ANTHROPOMETRY :

    Weight

    Length / Height

    Mid-arm circumference

NOTE :

INVESTIGATIONS

DATE

BLOOD :

Haemoglobin

T.L.C.

D.L.C.

Total Serum proteins

Serum albumin

Serum globulins

AG Ratio

URINE :

STOOL :

X-ray chest
(if necessary) :

IMMUNOLOGICAL
INVESTIGATIONS :

Total Haemolytic
complement
(CH_{50}) titre :

Alternative Pathway
Activity (AP_{50}) :

Serum Complement
C3 level :

Other Relevant Investigation :